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THE FRIEDMAN RABBIT OVULATION TEST IN DIFFERENTIAL OBSTETRIC DIAGNOSIS*

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ONE of the notable recent advances in medicine has been the development of the biologic tests for pregnancy based on the fundamental discoveries of Smith, Evans, Long, Simpson, Aschheim, Zondek, and others. In this country the original Aschheim-Zondek test has largely been supplanted by the simpler modification of Friedman.[1] The principles and technic are well enough known to need no further description here, but although the results of the original Aschheim-Zondek test have been analyzed by many authors, there has been no recent compilation and discussion of the Friedman test. And yet an understanding of the limitations as well as the value of this biologic method of diagnosing pregnancy is essential for its intelligent use by the many physicians to whom it is now available.

In addition to the new cases added here, the literature through March, 1933, has been thoroughly combed, and every report listed in the *Quarterly Cumulative Index Medicus,* with the exception of a very few in certain foreign languages, carefully analyzed. These have been studied for comparison of technic, percentage accuracy, the cause of false results, and the types of cases in which it has been used. A summary is presented in Table I.

In all, 5,225 cases have been presented by 34 authors, but for analysis sufficient details are given of only 4,515. The gross, uncorrected error is 3.9 per

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cent. The false negative findings have been divided into three sorts: 82 due to limitations of the test, 13 due to technical flaws admitted by the authors, and 54 that cannot be explained. The latter group, together with the 26 false positive findings, amount to 1.8 per cent.

A positive reaction is expected if a suitable rabbit is injected with a quantity of urine containing a sufficient amount of the prehypophyseal hormone. This hormone is generally considered to be elaborated with explosive suddenness shortly after fecundation. Positive tests have been reported as early as ten or twenty days after the known fruitful intercourse. In the author's series the earliest was in a woman with great irregularity of menses, who delivered a seven-pound child 260 days after the test was made. On the other hand, at least 48 cases have been reported (including 3 of this series) where early tests were negative, while later tests have proved correctly positive. The explanation may be that the prehypophyseal stimulation did not occur to the usual extent or else the eliminative threshold of the kidney was different than that usually found. In 7 cases reported, the test remained negative until the fourth or sixth month. Hence, the first limitation of the correctly performed test is that *a negative reading does not rule out early pregnancy.*

TABLE I

SUMMARY OF CASES REPORTED IN THE LITERATURE

AUTHOR	TECHNIC (SEE TEXT)	NUMBER CASES	NUMBER FALSE POSITIVES	GROSS PERCENTAGE INCORRECT READINGS
Friedman-Lapham[2]	FL	92	0	0
Martins[3]		100		
Magath-Randall[4]	MR	85	1	2.4%
Beasley[5]		45	0	2.2%
Rhamy[6]	FL(24 hr.)	56	0	1.8%
Dorn, et al.[7]	S	120	0	2.5%
Wilson-Corner[8]	WC	198	1	3.5%
White-Severance[9]	FL	40	0	10.0%
A. Brouha[10]	B	171	0	3.5%
Brindeau-Hinglais[11]	B	32	0	10.0%
Davis[12]	S	200	4	3.0%
Stricker[13]	RS	75	0	0
Grant, et al.[14]	RS	100	1	4.0%
Mathieu, et al.[15]		110		
Wood[16]	W	95	0	3.2%
Kincaid-Rafn[17]	MR	45	3	13.0%
Schoeneck[18]	RS-MR-S	123	0	13.0%
Parache[19]	FL	180	0	0.6%
Gustafson-Banks[20]		50	0	16.0%
Schneider[21]	S	500	0	0.4 plus %
Buettner[22]	S	100	2 ?	3.0%
Todd[23]	W (mod)	100	0	1.0%
Russum[24]	MR	40	1	10.0%
Sharp-Flohr[25]		73	1	1.4%
Wilson-Blanchet[26]	S-FL	250	2	1.0%
Ware-Main[27]	S	100	0	2.0%
Johnson-Townsend[28]	RS	24	1	16.0%
Reinhart[29]	RS	885	5	2.3%
Sondern-Silverman[30]	S	487	0	6.7%
Sharpley[31]	S (mod)	38	0	0
Litterer[32]	FL	140	1	2.7%
Mann, et al.[33]	FL	150	2	2.0%
King	RS-S	86	1	8.2%

In the differential diagnosis between *simple amenorrhea* and *pregnancy*, the author's series includes 40 cases, in all but 4 of which clinical diagnosis was difficult. There were three false negative responses at seven, twelve, and sixty days after the missed period, all later positive. There were 16 correct negative and 21 correct positive diagnoses, including two decisions between subinvolution and early pregnancy before the resumption of menses.

Once pregnancy is established the test will remain positive only as long as live chorionic tissue is in biologic contact with the mother. This must be borne in mind in using the test to diagnose abortion or intrauterine death. There are 107 such cases available for comparison. Of 14 *threatened abortions*, all gave positive reactions except 2 of the author's 5 cases. Both of these began bleeding at three months' gestation; from 5 to 8 c.c. of urine were used and the tests were read in twenty-four hours as negative; in both the bleeding was controlled and the pregnancy continued, positive readings being obtained subsequently. It is suggested that a sudden diminution in the amount of prehypophyseal hormone may have accounted for both the threatened abortion and the negative reactions.

Of *incomplete abortions* the literature describes 37, of which 20 gave positive reactions and 17, negative, in the latter group falling the author's 5 cases. Unfortunately the condition of the placenta is not stated in most of the reports. Two of the cases in the present series deserve some mention; one was seven months postpartum, the other twelve months, and each had bled for some two months previously. The tests were negative, and the pathologist's reports were "placental polyp" and "infected products of conception." Whether these women became pregnant subsequent to their deliveries and then aborted is not known.

In *complete abortion*, of which there are 39 cases, the same conditions hold which apply to the puerperium, namely that from twenty-four to seventy-two hours after delivery either a negative or a positive response may be expected.

Missed abortion may be divided into those before the fourth month and those after. Of 8 early cases in the literature, including one of the author's, all gave negative responses. After the fourth month death of the fetus may take place independent of damage to the placenta or its hormone capacity, and hence it is not surprising that Wilson and Corner[8] and Schoeneck[18] each report two positive reactions. A. Brouha[10] obtained two negative reactions, and Ware and Main[27] one. The author has four to add. In three, fetal death between the sixth and seventh month was diagnosed clinically, but positive reactions were obtained seven, seventeen, and thirty days, respectively, after the cessation of fetal movements. In the fourth case a five months' fetus had been dead three months and the rabbit response to the urine was negative. Thus a second limitation of the test is that *a positive reaction does not rule out death of the fetus.*

In *ectopic pregnancy* the test again must be used with discrimination. A positive reading indicates that live chorionic tissue has been in existence within the last seventy-two hours; a negative test means little. Of 57 ectopic pregnancies reported, only 41 gave positive reactions, of which 2 are added in this series. It may be assumed that in the others either the hormone concentration was not sufficient or else the chorionic tissue had been long dead.

On the other hand, in hydatid mole and chorionepithelioma perfect satisfaction is expressed in all reports.

Cancer of the uterus, while occasionally giving a positive Aschheim-Zondek test, is usually reported as giving a negative Friedman reaction. One case in this series was negative. But another woman, aged thirty-eight, who had had her last child four years previously and had been bleeding irregularly for two years, passed some tissue, which from description might have been a mole. Two weeks later her Friedman test was positive. She was curetted, and the removed tissue diagnosed by the pathologist as "acanthoma" (squamous cell carcinoma). Two weeks later the Friedman test was again positive. She had a stormy convalescence but recovered, and two months later appeared to be in fair health. This case is presented as the only false positive in the series. The literature includes two other cases of carcinoma which gave positive, although atypical, responses.

In patients of obese habitus it is often difficult or almost impossible to differentiate *fibroids or ovarian cyst from pregnancy,* and where amenorrhea supervenes the Friedman test becomes particularly valuable. In this series there were 13 such cases of amenorrhea where the physician was frankly doubtful of the diagnosis and the test proved subsequently to be correct: 4 at two months, all negative; 5 at three months of which 1 was a case of pregnancy; and 4 at four months of which 2 were cases of pregnancy.

Of the greatest importance is learning of the existence of *pregnancy in the presence of a fibroid or an ovarian cyst* before laparotomy. In 6 cases in this series amenorrhea led the gynecologist to use the Friedman test before operating; four were correctly negative, and two correctly positive. In one of these two cases hysterectomy for fibroids was postponed until term; in the other removal of a dermoid cyst was not done until pregnancy had advanced to a point where the safety of the fetus was not threatened.

Other differential diagnoses in this series consisted of: menopause against pregnancy, pelvic inflammatory disease against pregnancy, and finally a case of menorrhagia in a girl of 18 (with a negative test) later shown to be puberty hyperplasia. Table II summarizes the cases in this series in which, with a few exceptions, the test was used to aid the author or other physicians to decide between several possible diagnoses.

It must be borne in mind that many of the false results may have been due to the method of performing the test. Seven different technics with minor variations are described in the literature. These are: that of Friedman and Lapham[2] ("FL"), where 5 c.c. of urine are injected three times a day for two days and the ovaries examined in forty-eight hours; that of Magath and Randall[4] ("MR") who give 15 c.c. and operate in thirty hours; that of Reinhart and Scott[29] ("RS") where a single injection of 5 c.c. is followed by examination in twenty-four hours; that of Wilson and Corner[8] ("WC") who laparotomize the animal in sixteen hours after a 5 c.c. injection; that of Schneider[21] ("S") who uses a larger dosage and waits forty-eight hours; that of A. Brouha[10] ("B") who opens the rabbit before injecting 5 c.c. and makes a final reading in twenty-four hours; and that of Wood[16] ("W") who laparotomizes first, gives 10 c.c., laparotomizes in twenty-four hours, and if negative gives another 10 c.c. and makes a final reading the next day.

The experience of men working with hormone reactions in lower animals indicates that frequent small injections of endocrine substances is far more effective than a single large dose. Friedman and Lapham's 92 cases without an error tends to confirm this observation. While Wilson and Corner obtained excellent results with sixteen-hour readings, many workers including the author have found that the reaction frequently does not become positive until between the twenty-fourth and forty-eighth hour. It is for this reason that although Reinhard and Scott's technic was used at first, in the latter cases of this series 10 or 12 c.c. in a single injection was followed by laparotomy in forty-eight hours as the most desirable routine.

TABLE II

SUMMARY OF 103 TESTS ON 86 WOMEN FOR DIAGNOSIS OF CHORIONIC TISSUE

	CASES	POSITIVE	NEGATIVE
Pregnancy vs. Amenorrhea	24 16	21 0	3 16
Threatened abortion Incomplete abortion	5 5	3 0	2 5
Early fetal death Later fetal death	1 4	0 3	1 1
Ectopic pregnancy	2	2	0
Cancer	2	1	1
Fibroids or cyst vs. pregnancy	12 3	0 3	12 0
Tumors with, vs. without pregnancy	2 4	2 0	0 4
Menopause P.I.D. Menorrhagia	3 2 1	0 0 0	3 2 1

It is not unlikely that the breed of rabbit and the climate or season determines whether or not ovulation is quickly produced. The size of the rabbit is of equal importance. The lower limits as given by most authors are 1.5 kg., ordinarily three and one-half months of age. Adult rabbits are widely used after proper isolation even from other females. While Davis[12] considers fourteen days sufficient (but reports 4 false positive reactions) twenty-one to twenty-eight days is usually held to be the minimum. Death of the rabbit from a toxic factor in the urine is reported by six different authors as occurring in 2.7 per cent, 2.7 per cent, 5.8 per cent, 5.8 per cent (this series), 8.9 per cent, and 12 per cent. The cause of death, be it too acid or too cold a urine, or some poisonous substance has not been determined. Simple anaphylaxis, however, seems to have been ruled out.

As Snyder and Wislocki[34] have pointed out, the Friedman test is specific for the human female, and hence is not applicable to veterinary purposes. Ignorance of this fact recently led a well-known gynecologist to condemn the test in general for its failure to indicate pregnancy in three of his mares. On the other

hand the work of White and Severance[9] shows that in other respects the test is not as delicate as that of L. Brouha on male mice.

Finally there must beconsidered what constitutes a positive reaction. It is agreed that a translucent follicle, without color, is a negative response. Wilson and Corner[8] using a sixteen-hour technic describe a freshly ruptured, bleeding, red follicle as a true positive, and warn against a small, dark brown, rather conical body occasionally seen. Most authors look for a large, rounded, purplish corpus hemorrhagicum. Buettner[22] reports as a true positive a large pale unruptured follicle with tiny red flecks. Laparotomy within a week of three such observations showed the structures to have formed true corpora lutea. Grant[14] also observed such a follicle in one case which he reported as positive. Definite decision can be made by microscopic examination which will reveal beginning luteinization. Gustafson and Banks[20] report 5 cases grossly negative but correctly positive after histologic study. In the author's series a similar instance is recorded.

The cause of false positive responses is still to be worked out. Table III summarizes the false positive cases according to the cause or the diagnosis. Aside from the technical errors in handling the rabbits, any condition which might cause stimulation of the anterior lobe of the pituitary gland might be expected to cause a development of *prolan* which may be reflected in a positive rabbit reaction. Kraus[35] reports that an increase in *prolan* may be the result of elevated intracranial pressure. Germ cell tumors, pituitary gland tumors, compensatory hypertrophy of the pituitary gland, must all be considered.

The Friedman test for the diagnosis of pregnancy or its products is rapidly becoming a part of the physician's equipment along with the Wassermann test, agglutination reactions, and the leucocyte count. But it must retain its proper relationship to history taking and physical diagnosis. An interesting experience of one of the leading gynecologists of the country emphasizes its usefulness: his

TABLE III

TABULATION OF FALSE POSITIVE REACTIONS

Details not given	9	
Uncontrolled rabbit	2	
Improper segregation	2	
Puberty bleeding	1	Wilson and Corner
Castration	1	Sharp-Flohr
Teratoma testis	1	Russum
Carcinoma	3	Grant, et al. Buettner King
Unexplained	7	

diagnosis of a case was that of a prolapsed twisted ovarian cyst, while his assistant was convinced that the patient had a recently ruptured ectopic pregnancy. The Friedman test was positive; laparotomy revealed on one side the tubal pregnancy and on the other side a small cyst which had prolapsed into the culdesac and which he had felt. But on the other hand, teachers in all branches of medicine find that their students, instead of training themselves to detect

pregnancy by their five senses, depend on a rabbit test to make the diagnosis. This test, however valuable, should never become popular at the expense of the *Tactus eruditus.*

CONCLUSIONS

1. The results of 103 Friedman rabbit ovulation tests on 86 women are reported and discussed.

2. The literature to date is reviewed. Of 4,500 cases, the gross error has been 3.9 per cent; the corrected error, including the 26 false positive readings, amounts to 1.8 per cent.

3. The test is a valuable aid in differential diagnosis, provided its limitations are recognized.

4. A positive test indicates usually, but not always, the presence of live chorionic tissue. It may not become positive until pregnancy is well advanced, and if positive may not necessarily mean that the fetus is alive.

5. The test should assist but not supplant the usual diagnostic technic.

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THE RELATIONSHIP BETWEEN THE CORONARY BLOOD SUPPLY TO EXPERIMENTALLY PRODUCED VENTRICULAR LESIONS AND THE RESULTING ELECTROCARDIOGRAPHIC ALTERATIONS*

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IN A RECENT publication[1] the authors reported the electrocardiographic changes following ventricular myocardial lesions produced by the electric cautery. Particular attention was directed to the R-T segment and T-wave alterations resulting from definitely placed lesions, the localization of which was related to certain electrocardiographic characteristics as classified by Parkinson and Bedford.[2] It was found that cauterization of the anterior surface of the left ventricle gave curves of the T_1 type while a similar lesion of the posterior surface of this ventricle and of any portion of the right ventricle (except at the base anterior where no change was observed) resulted in curves of the T_3 type. Substantially similar results have been obtained recently by Haney, Borman and Meek[3] who investigated the changes in the R-T and S-T segments of the electrocardiogram in dogs in which the myocardial lesions were produced by radon implants.

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This report is concerned with the relationship between the blood supply (as from the right or left coronary artery) to well-defined ventricular areas damaged by the electric cautery and the resulting electrocardiographic alterations. In a series of twelve cats, the coronary arteries were injected, by a method previously described,[4] and the vessel distribution, with respect to the damaged areas, studied in detail.

TABLE I

COMPARISON BETWEEN THE SITE BURNED, THE TYPE OF CURVE PRODUCED AND THE BLOOD SUPPLY

NO.	SITE BURNED	TYPE	LEFT CORONARY	RIGHT CORONARY	MOSTLY LEFT CORONARY	MOSTLY RIGHT CORONARY
3	Left apex ant.	T_1	+			
6	Left apex post.	T_3	+			
7	Left base post.	T_3				+
30	Left apex ant.	T_1	+			
31	Left apex post.	T_3			+	
32	Right apex ant.	T_3			+	
33	Left base post.	T_3			+	
34	Left apex post.	T_3	+			
35	Left base post.	T_3	½	½		
36	Right apex post.	T_3			+	
37	Right base post.	T_3				+
38	Right base post.	T_3		+		

RESULTS (TABLE I)

Anterior Surface of the Left Ventricle.—Two specimens (No. 3 and No. 30) were studied, in both of which the lesion had been produced at the apex and a T_1 type of curve obtained. In each instance the whole of the damaged area was supplied by the left coronary artery.

Posterior Surface of the Left Ventricle.—Six hearts, in all of which the resulting curve had been of T_3 type, were injected. In three the lesion was at the apex. Of these the left coronary artery supplied the whole of the injured area in two (No. 6 and No. 34) and the greater part of it in the third (No. 31). In the other three, the damage was at the base, the left coronary being the principal supply in one (No. 33), the right coronary in another (No. 7), while both played an equal part in the third (No. 35).

Anterior Surface of the Right Ventricle.—One heart (No. 32), in which a lesion had been produced at the apex and a T_3 type of curve obtained, was studied. The left coronary artery was the principal supply to this area.

Posterior Surface of the Right Ventricle.—This series consisted of three hearts, in which all of the curves were of the T_3 type. In one instance (No. 36), the damaged area was situated at the apex with the left coronary artery as the chief source of supply. In the other two, in which the lesions were at the base, the right coronary artery supplied the whole region in one (No. 38) and the greater part of it in the other (No. 37).

To summarize: It was found that in the two experiments in which a T_1 type of curve was obtained, the damaged site was supplied in whole by the left coronary artery. In the instances in which a T_3 type of curve resulted, the coronary distribution varied considerably. The area involved was sup-

plied wholly by the left coronary artery in two cases and in great part in four others. In the remainder, the right coronary artery supplied all of the cauterized region in one, was the main supply in two, while in another both vessels appeared to be of equal importance.

DISCUSSION

Barnes and Whitten[5] found that in infarcts of the anterior portion of the left ventricle, either alone or combined with a similar lesion at the apex, curves of the T_1 type were produced; whereas in those of the posterior surface, with or without involvement at the apex, the tracings were of the T_3 type. They believed that it was probably more than a coincidence that the plane of division which decided the type of electrocardiogram should follow the average plane of separation between the blood supply from the right and left coronary arteries. Despite this they suggested that it was the site of the lesion rather than the particular blood supply which determined the changes in the R-T interval, but that the site would usually indicate the artery involved. Bell and Pardee[6] analyzed five cases of their own and seven of Levine's series[7] and found that thrombosis of the left coronary artery was associated with changes of T_1 type, while occlusion of the right coronary artery gave tracings of T_3 type. They stated: "We therefore feel that in the absence of bundle-branch block, the electrocardiogram may be used in localizing the thrombus in either the right or left coronary system. We cannot offer any particular explanation for this relation between the vessel occluded and the particular T-wave that is inverted." On the other hand, Gilchrist and Ritchie,[8] as a result of a study of two cases of their own and a large series from the literature, concluded that changes in the R-T segment were strong presumptive evidence of myocardial infarction, but they did not believe the data available sufficient to warrant the view that the form of the electrocardiographic change could be considered a definite localizing sign of the infarct. Barnes and Mann[9] investigated the question in dogs surviving for varying periods, electrocardiograms being taken at intervals until death, when autopsies were performed. These workers tied off branches of the left coronary artery, supplying the posterior surface of the left ventricle, and obtained T_1 type of curves, ligation of the right coronary artery resulting in T_3 type. When one considers that a T_1 type was obtained when the left ventricle was infarcted and a T_3 type when a similar lesion was produced in the right ventricle, the fact that in the dog the left coronary artery supplies the whole of the left ventricle and the right coronary, nearly the whole of the right[10] might assume some importance in establishing a relationship between the type of RS-T alteration and the blood supply. Despite their findings, these authors do not consider the latter to be the determining factor, since they state that in man the electrocardiographic changes following acute infarction of the posterior basal portion of the left ventricle are constant regardless of whether this region is supplied by the right or left coronary artery. Wilson and his collaborators,[11] on correlating the form of the electrocardiogram with the location of the infarct in the human heart, state that the Q_1T_1 type is usually asso-

ciated with a lesion of the anterior surface of the left ventricle, the Q_3T_3 with one of the posterior surface. In some instances, however, lesions of these sites did not produce any characteristic change in the electrocardiogram. Fowler, Rathe and Smith[12] in a series of survival experiments in dogs, ligated small branches of the coronary arteries, both right and left, and found mainly T-wave changes. They were unable to correlate the location of the infarct and the type of electrocardiogram produced. Our results in the cat show that on the anterior surface of the left ventricle, the blood supply was always from the left coronary artery, and the electrocardiograms, following the production of localized lesions, were of the T_1 type. Over the entire surface of the heart posteriorly, T_3 types of curves were obtained, despite marked variations in blood supply. When one studies the relationship of all the T_3 type of curves to the blood supply of the damaged areas without consideration of the region of the heart involved, the results appear grossly inconsistent. This view is further substantiated by an examination of the normal coronary distribution in the cat. In a recent study dealing with the latter,[4] it was found that posteriorly, in 65 per cent of cases the right coronary artery supplied all of the right ventricle and varying portions of the left ventricle, ranging from a small strip adjoining the posterior interventricular sulcus to almost one-half of the left ventricle. The remainder of this ventricle received its supply from the left coronary artery. In the other 35 per cent of cases the left coronary artery supplied posteriorly all of the left ventricle and the adjoining part of the right ventricle, while the right coronary in these hearts supplied only a small portion of the right ventricle.

In view of these anatomical observations we believe that the coronary distribution in the cat resembles that in man to a greater degree than does that in the dog. Although on the anterior surface of the heart, the subbranches of the right and left coronary arteries show a generally similar arrangement in all three, posteriorly there are some marked differences. In man, according to Gross[13] and also Barnes and Whitten,[5] the right coronary artery in the majority of cases gives off a posterior descending branch which extends down the posterior interventricular sulcus; the right coronary artery, itself, continuing on to the left ventricle to supply a considerable part of the latter adjacent to the sulcus. In these cases the circumflex branch of the left coronary plays an unimportant rôle in supplying the posterior surface of the heart. In a small percentage of cases, the situation is reversed, the circumflex reaching the posterior sulcus and continuing down in it, while the right coronary artery terminates soon after it reaches the posterior surface of the right ventricle. In these instances, posteriorly the whole of the left ventricle and the adjoining portion of the right are supplied by the left coronary artery. In the dog, according to Moore[10] the right coronary artery never reaches the posterior interventricular sulcus, but ends at some point between it and the right border of the heart. In all cases, the circumflex division of the left coronary artery proceeds down the posterior sulcus as the posterior descending division, and along with its other branches, supplies the whole of the left ventricle posteriorly.

In man, the septum in its basal two-thirds is supplied by penetrating branches of the anterior and posterior descending arteries, subdivisions usu-

ally of the left and right coronary arteries respectively. The apical portion is supplied entirely by twigs from the anterior descending artery. In the case of the dog, branches of the septal division of the left coronary artery anastomose with penetrating vessels from the anterior and posterior descending arteries, the whole of the septum thereby being supplied by the left coronary artery. In the cat, in the majority of cases, the septum receives its supply from penetrating branches of both right and left coronary arteries, while in the remaining hearts, wholly from branches of the latter.

The R-T segment alterations associated with myocardial damage after blood vessel occlusion or direct injury are, we think, the expression in a limb lead of a monophasic variation of the demarcation current set up in the heart as the result of a potential difference between the injured and the adjacent uninjured areas. One should not expect that thrombosis of a particular coronary artery, per se, could produce the definite change observed in the electrocardiogram in coronary thrombosis. Only in the case of a constant blood supply would a characteristic change in the electrocardiogram be related to the occlusion of a particular artery. In our experiments on the cat, the blood supply (as to right or left coronary arteries) to the posterior surface of the heart varied markedly, and still on cauterization of any portion of this site a T_3 type of curve was obtained in every instance. When we consider as well that the anatomical studies have pointed to a close resemblance between the coronary blood supply in man and that in the cat, it strengthens our belief that in the former it is hazardous to diagnose a thrombus in one or the other coronary vessel in a T_3 type of curve. With a T_1 type there appears to be every probability that the lesion is located in the left coronary artery. The final decision on this question in man must await the careful study of a sufficient number of hearts in which the electrocardiographic and pathologic data have been investigated and correlated. On the other hand, since all authors agree that infarct of the right ventricle is rare, it seems to us probable that a definite T_1 type of electrocardiogram indicates a lesion of the anterior surface of the left ventricle and a T_3 type one of its posterior surface.

SUMMARY AND CONCLUSION

The relationship between the blood supply (as from the right or left coronary artery) to experimentally produced ventricular lesions and the resulting electrocardiographic alterations was investigated in a series of injected cats' hearts.

Although on the anterior surface of the heart, there appeared to be a relationship between the curves and the blood supply, on the posterior surface, both of the right and left ventricles, tracings of T_3 type were produced, whereas the blood supply to these areas varied markedly.

The results support the view that the characteristic T-wave changes are not constantly related to the occlusion of a particular coronary artery.

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THE EFFECT OF HYPERPYREXIA UPON THE PHOSPHORUS PARTITION OF WHOLE BLOOD IN PARESIS*

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RECENT investigations have shown there are changes in the amount of the various phosphorus fractions of the blood of neurosyphilitic patients following treatment. Rosen, Krasnow, and Notkin[1] have found a distinct increase in the amount of lecithin in the blood of syphilitic patients after treatment. Bischoff, Maxwell, and Hill[2] and Daly and Knudson[3] have demonstrated that there is a shift in the phosphorus equilibrium during hyperthermia.

PLAN OF EXPERIMENT

The results of the work cited above would indicate that the treatment of paresis with hyperpyrexia might have a definite influence upon the phosphorus partition of the blood. This problem was approached by the following procedure: Fifteen patients diagnosed as typically paretic were chosen for this study; nine were treated by the electric blanket method[4] and six with inoculated tertian malaria. A phosphorus partition, involving inorganic, total acid-soluble, organic acid-soluble, lipin, and total phosphorus, was determined upon the whole blood of each patient before and after the treatment series. Those treated by the electric blanket method each received twelve treatments, and those who were given malaria were each allowed to have five paroxysms.

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The blood for analysis was taken in the morning after a fifteen-hour fast, and the patients, while undergoing the series of treatments, were kept under carefully controlled hospital conditions.

The methods of analysis used in this study were those recommended by Walker and Huntsinger.[5] Each determination was done in duplicate and checked independently by two workers. Mean values were used when duplicate determinations did not agree.

TABLE I

PHOSPHORUS PARTITION OF WHOLE BLOOD IN PARETIC PATIENTS BEFORE AND AFTER TREATMENT WITH HYPERPYREXIA

CASE	1 TOTAL ACID-SOLUBLE P (MG. PER 100 ML.)	2 INORGANIC P (MG. PER 100 ML.)	3 ORGANIC ACID-SOLUBLE P (1 MINUS 2) (MG. PER 100 ML.)	4 LIPIN P (MG. PER 100 ML.)	5 TOTAL P BY ANALYSIS (MG. PER 100 ML.)	6 TOTAL P BY ADDITION (1 PLUS 4) (MG. PER 100 ML.)	CONDITION OF PATIENT
1							
Before	21.79	3.25	18.54	10.63	33.33	32.42	
After	19.61	3.65	15.96	12.04	33.33	31.65	Improved
2							
Before	26.31	2.62	23.69	11.07	36 27	37.38	
After	25.00	3.27	21.73	12.20	36.86	37.20	Improved
3							
Before	23.88	1.63	22.25	11.33	35.02	35.21	
After	20.20	3.50	16.70	11.87	32.56	32.07	Improved
4							
Before	22.47	2.66	19.81	12.15	31.37	34.62	Not
After	20.72	3.12	17.60	11.87	32.56	32.59	Improved
5							
Before	24.84	3.32	21.52	12.87	35.49	37.71	Not
After	20.44	2.53	17.91	12.12	32.26	32.56	Improved
6							
Before	25.71	3.26	22.45	11.43	34.84	37.14	
After	21.62	2.54	19.08	12.05	34.04	33.67	Improved
7							
Before	26.49	3.88	22.61	13.97	40.81	40.46	
After	27.58	3.20	24.38	14.81	42.55	42.39	Improved
8							
Before	26.14	3.24	22.90	12.00	36.36	38.14	
After	30.76	2.92	27.84	14.13	43.71	44.89	Improved
9							
Before	23.81	3.46	20.35	10.52	34.93	34.33	
After	23.53	2.96	20.57	12.87	36.03	36.40	Improved
10							
Before	25.62	3.15	22.47	14.30	40.55	39.92	
After	24.07	2.90	21.17	14.81	39.02	38.88	Improved
11							
Before	29.62	3.11	26.51	13.11	42.23	42.73	
After	25.74	2.81	22.93	14.61	39.02	40.35	Improved
12							
Before	30.01	3.72	26.29	12.69	42.78	42.70	
After	24.84	3.91	20.93	14.29	38.64	39.13	Improved
13							
Before	28.98	3.65	25.33	13.33	42.75	42.31	
After	23.52	2.87	20.65	14.54	37.20	38.06	Improved
14							
Before	29.19	3.90	25.29	14 35	43 24	43.54	
After	27.58	3 55	24.03	15.50	43.53	43.08	Improved
15							
Before	23.12	3 63	19.49	14.44	38.08	37.56	
After	26.13	3.90	22.23	15.09	40.00	41.22	Improved

RESULTS

The data incorporated in Table I give the findings of the various phosphorus partitions before and after treatment. The accuracy of the analytic methods employed is demonstrated by the close agreement between the total phosphorus by direct analysis and the sum of the acid-soluble and lipin fractions. This table also gives the status of each patient following treatment.

Table II gives the amount of lecithin per 100 ml. of whole blood for each patient before and after treatment. These figures were obtained by multiplying

TABLE II

LECITHIN CONTENT OF WHOLE BLOOD IN PARETIC PATIENTS BEFORE AND AFTER TREATMENT WITH HYPERPYREXIA

CASE	BEFORE (MG. PER 100 ML.)	AFTER (MG. PER 100 ML.)	CONDITION OF PATIENT AFTER TREATMENT
1	265.75	301.00	Improved
2	276.75	305.00	Improved
3	283.25	296.75	Improved
4	303.75	296.75	Not improved
5	321.75	303.00	Not improved
6	285.75	301.25	Improved
7	339.15	370.25	Improved
8	300.00	353.25	Improved
9	263.00	321.75	Improved
10	357.50	370.25	Improved
11	327.75	365.25	Improved
12	317.25	357.25	Improved
13	333.25	363.50	Improved
14	358.75	387.50	Improved
15	361.00	377.25	Improved

the lipin phosphorus by 25, as lecithin contains 4 per cent phosphorus. This gives recognition only to the lecithin content of this fraction, but this has been found accurate enough for comparative purposes. The status of each patient following treatment is also included in this table.

DISCUSSION

Bischoff et al.[2] have found that during hyperthermia part of the inorganic phosphorus in the blood is converted into acid-soluble organic phosphorus with an alkaline shift in the blood P_H. They have suggested that this is an in-vivo duplication of the in-vitro conversion of inorganic to organic phosphorus in blood as first demonstrated by Lawaczek.[6]

Daly and Knudson[3] in their experiment in hyperthermia were unable to confirm the observation of Bischoff and his coworkers that the alkalosis occurring during hyperthermia was accompanied by an increase in the acid-soluble organic phosphorus and a decrease in the inorganic fraction. They found that there was a lowering of both inorganic and acid-soluble organic phosphorus of whole blood and plasma during alkalosis.

It was also shown by Rosen et al.[1] that patients with neurosyphilis, who improve following treatment, show an increase in the lecithin content of their blood.

Referring to Table I and excluding patients 4 and 5, who did not improve following treatment, a lowering of the inorganic phosphorus content is shown in eight of the thirteen improved cases. Again nine of these thirteen patients had a loss of organic acid-soluble phosphorus. This table also shows that all of the thirteen improved cases had an increase in the amount of the lipin phosphorus fraction.

The above facts are not in perfect agreement with the findings of either Bischoff or Daly and Knudson. This perhaps can be explained by the fact that their analyses were done during the period of hyperthermia while samples analyzed in this problem were taken at the normal body temperature before and after the treatment series. However, the results of the majority of our analyses agreed with the findings of Daly.

There is evidence that the synthesis of phosphatides from inorganic phosphates can take place in the body.[7] Animals have been shown to develop normally when their only source of phosphorus was in the inorganic form, and to be capable of forming both organic phosphorus for their bodies and lecithin for their eggs. It is significant to note, therefore, that in nine of the thirteen improved cases there was an increase in the lipin fraction accompanied by a lowering of both the total acid-soluble and organic acid-soluble phosphorus. This might be used as evidence that part of the acid-soluble fraction had been converted into lipin phosphorus as a result of the hyperpyrexia.

Regarding the total determined phosphorus in Table I, it will be noticed that there were no significant changes in the amount following treatment in the thirteen improved cases. It increased in five, decreased in seven and remained constant in one. This would suggest that the increase in the lipin phosphorus was not caused by an increase in the total amount of phosphorus in the blood, but represented only a shift from one fraction to another.

The only significant change observed in the unimproved Cases 4 and 5 was that there was a decrease in the amount of lipin phosphorus after treatment. This may prove to be indicative, but two cases can hardly be considered as sufficient evidence to arrive at any definite conclusion.

Table II shows that there was an increase in the lecithin content of the blood of the improved cases. This agrees with the findings of Rosen et al.[1]

CONCLUSIONS

1. Paretic patients who improved following treatment by hyperpyrexia showed an increase in the lecithin content of their blood.

2. There seemed to be a conversion of acid-soluble phosphorus to lipin phosphorus following treatment by hyperpyrexia in those cases which improved.

3. The increase in the amount of lipin phosphorus in the blood of improved patients following hyperpyrexia was not accompanied by a corresponding increase in the total determined phosphorus, and would seem to be a partitional change.

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SPINAL FLUID SUGAR DETERMINATIONS IN EXPERIMENTAL HYPOGLYCEMIA OF DOGS*

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RECENTLY one of us (R. D.) had occasion to treat an unusual case of severe insulin shock. The patient, a man aged thirty-one years, was a known diabetic patient and, while under the influence of alcohol, took 30 units of insulin before the evening meal, which was twice his prescribed dose. He promptly vomited and members of his family thinking he was going into a diabetic coma administered two more 30-unit doses of insulin. The patient became more stuporous during the night and was sent to the hospital in the morning.

On admission his blood sugar was 21 mg. per 100 c.c. A total of 100 gm. of glucose was given intravenously, but the patient did not regain consciousness though the blood sugar was then 164 mg. per 100 c.c. Intravenous injections of glucose were continued but the patient died without regaining consciousness. The blood sugar after death was 176 mg. per 100 c.c., but the spinal fluid sugar was only 25 mg. per 100 c.c.

It is difficult to evaluate the rôle played by alcohol in this case but the discrepancy between blood and spinal fluid sugar results made it desirable that a study be made of the blood and spinal fluid sugar in experimental hypoglycemia in the hope that the poor response of such patients to large amounts of injected glucose might be explained.

EXPERIMENTAL

Normal dogs of both sexes and various weights were used. Insulin was administered after a twenty-four-hour fasting period. Blood was taken from the jugular vein and spinal fluid obtained by cisternal puncture without anesthesia. The specimens were preserved with sodium fluoride and the glucose determinations made by the method of Folin and Wu.[1]

Table I lists the blood and spinal fluid sugar findings of five normal dogs. It is generally agreed that in a state of health the blood and spinal fluid sugar are in fairly definite relationship to each other. However, we are unaware of

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any simultaneous determinations made on the experimental animal. Kasahara and Uetani[2] though giving figures for spinal fluid sugar following insulin injections did not report simultaneous blood sugar values, and the relationship of the two values were only assumed. In humans this ratio is approximately 4:3 which corresponds closely to what we have found in dogs.

TABLE I

BLOOD AND SPINAL FLUID SUGAR OF NORMAL DOGS

DOG NO.	BLOOD SUGAR MG. PER 100 C.C.	SPINAL FLUID SUGAR MG. PER 100 C.C.	RATIO
1	91	68	1.34
2	84	65	1.29
3	81	60	1.35
4	108	73	1.48
5	82	64	1.28
Average	–	–	1.35

TABLE II

EFFECT OF INSULIN HYPOGLYCEMIA ON SPINAL FLUID SUGAR

DOG NO.	FASTING BLOOD SUGAR (MG. PER 100 C.C.)	FASTING SPINAL FLUID SUGAR (MG. PER 100 C.C.)	INSULIN	"SHOCK" BLOOD SUGAR (MG. PER 100 C.C.)	"SHOCK" SPINAL FLUID SUGAR (MG. PER 100 C.C.)	RATIO
1 8 kg.	11:20 A.M. 91	11:20 A.M. 68	11:30 A.M. 80 units (subcutaneously)	1:40 P.M. 25	1:45 P.M. 24	1.04
2 11 kg.	10:40 A.M. 84	10:40 A.M. 65	10:55 A.M. 110 units (subcutaneously)	12:55 P.M. 37	1 P.M. 23	1.61
3 10.4 kg.	4:15 P.M. 82	4:20 P.M. 64	4:25 P.M. 20 units (subcutaneously)	6 P.M. 44	6:05 P.M. 39	1.14
4 11.8 kg.	3:40 P.M. 108	3:55 P.M. 73	4 P.M. 59 units (subcutaneously)	5:30 P.M. 51	5:35 P.M. 41	1.24
5 11.8 kg.	1:20 P.M. 81	1:28 P.M. 61	1:30 P.M. 59 units (intraspinally)	3:05 P.M. 61	3:15 P.M. 32	1.91
6 10.3 kg.	–	–	11:45 A.M. 60 units (subcutaneously)	1:15 P.M. 34	1:20 P.M. 28	1.21
7 10.2 kg.	–	–	9:55 A.M. 80 units (subcutaneously)	11:55 A.M. 44	12 N 33	1.33
7 10.2 kg.	–	–	10:40 A.M. 40 units (intraspinally)	12:15 P.M. 33	12:20 P.M. 23	1.44
8 12.6 kg.	–	–	9:55 A.M. 40 units 11:30 A.M. 80 units 12:35 P.M. 40 units 1:20 P.M. 40 units (subcutaneously) Total 200 units	2:10 P.M. 33	2:15 P.M. 17	1.94

In the production of hypoglycemia in dogs Drabkin and Shilkret[3] used 20 units of insulin per kilogram. In our work we used smaller doses, not considering it desirable to produce the severe hypoglycemia that is obtained with such large doses. Our dosage ranges were between 2 and 10 units per kilogram with one exception. Dog No. 8 received 15.8 units per kilogram. The criteria of shock were listlessness, apathy, unsteady gait, vomiting, and loss of control of bowels and bladder.

It will be noted from Table II that in no case did the spinal fluid fail to show a decided decrease in sugar following insulin administration. The two dogs receiving insulin intraspinally showed a somewhat greater proportional fall in spinal fluid sugar per unit of insulin but part of this excessive decrease might be accounted for by simple dilution of spinal fluid by the insulin. The blood and spinal fluid sugar relationship is definitely disturbed; in only one animal was the normal ratio maintained. It is also noteworthy that in very severe hypoglycemia (dog No. 8) the effect on the spinal fluid sugar is proportionally so much greater as to cause this ratio to approach two. Insulin is active intraspinally; the blood sugar findings, however, indicate that it is no more efficacious than when administered subcutaneously.

The influence of intraspinal glucose administration upon insulin hypoglycemia is illustrated in Table III.

After shock had been attained in the six dogs, varying amounts of glucose solution were injected intraspinally. Following each injection, the blood sugar, as well as the spinal fluid sugar, was determined in order to see if there was a rise in the blood value coincident with any improvement noted.

Mater[4] has reported a constant rise in the blood sugar following the injection of air and Ringer's solution into the subarachnoid space, amounting to 200 mg. per cent in some cases. Such a rise, however, could be suppressed by the preliminary use of insulin. Our results confirm these observations, the blood sugar rising but little following intraspinal glucose injections in the insulin-treated dogs.

In all our cases of intraspinal glucose injections the improvement noted was transient and difficult to evaluate. To eliminate the possible effect on the clinical improvement of the stimulation caused by performing a cisternal puncture, one animal was injected with normal salt solution. This injection was without effect (dog No. 6, Table III). Wagging the tail and loss of apathy were taken as criteria of improvement. Such changes were but temporary, and in order to save the animal at the end of the experiment it was necessary to administer glucose intravenously.

Dog No. 5 was given a very large dose of insulin and severe shock with convulsions was produced. Repeated intraspinal injections of glucose solution failed to ameliorate the symptoms despite the fact that the spinal fluid sugar reached a level more than twice normal.

During the course of this work it was interesting to note the rapidity with which glucose, injected intravenously, penetrated into the spinal fluid.

Thus following the injection of 25 c.c. of 50 per cent glucose in seven minutes the spinal fluid sugar of dog No. 5 rose from a hypoglycemic value of 28 to 78 mg. per 100 c.c. Dog No. 6 showed a similar rise. However, such

TABLE III

THE EFFECT OF INTRASPINAL GLUCOSE INJECTIONS UPON INSULIN HYPOGLYCEMIA

DOG NO.	"SHOCK" BLOOD SUGAR (MG. PER 100 C.C.)	"SHOCK" SPINAL FLUID SUGAR (MG. PER 100 C.C.)	INTRASPINAL GLUCOSE	BLOOD SUGAR AFTER INTRASPINAL GLUCOSE (MG. PER 100 C.C.)	RESULT
1 8 kg. 80 units (subcutaneous) 11:30 A.M.	1:40 P.M. 25	1:45 P.M. 24	3 c.c. of 2% glucose 1:45 P.M.	32 1:55 P.M.	Dog lifted ears and began to wag tail at once. After 5 min. seemed to be back in shock. Was given 40 c.c. of 50% glucose intravenously. Dog died during the night.
6 10.3 kg. 60 units (subcut.) 11:45 A.M.	1:15 P.M. 34	1:20 P.M. 28	2 c.c. normal saline (intraspinally) 1:20 P.M.	37 1:25 P.M.	No change noted. Given 25 c.c. of 50% glucose intravenously. Dog survived.
2 11 kg. 110 units (subcut.) 10:55 A.M.	12:55 P.M. 37	1 P.M. 23	3 c.c. of 10% glucose 1 P.M.	41 1:05 P.M.	Little, if any improvement. Dog had to be given 40 c.c. of 50% glucose (intraven.) but showed poor response. Died at 6 P.M.
7 10.2 kg. 80 units (subcut.) 9:55 A.M.	11:55 A.M. 44	12 N. 33	2.5 c.c. of 4% glucose 12 N.	37 12:05 P.M.	Showed improvement for 15 min. but rapidly went back into shock and was given 25 c.c. glucose intravenously. Dog survived.
7 10.2 kg. 40 units (intraspin.) 10:45 A.M.	12:15 P.M. 33	12:20 P.M. 23	2 c.c. of 4% glucose 12:20 P.M.	38 12:25 P.M.	Dog improved temporarily but symptoms of shock followed. Survived after 25 c.c. of 50% glucose was given (intraven.).
8 12.6 kg. 9:55 A.M. 40 units 11:30 A.M. 80 units 12:35 P.M. 40 units 1:20 P.M. 40 units (subcut.) Total 200 units	2:10 P.M. 43 3:40 P.M. 50	2:15 P.M. 17 3:45 P.M. 160 4:40 P.M. 177	2:20 P.M. 2 c.c. 4% glucose 3:50 P.M. 2 c.c. 4% glucose 4:50 P.M. 2 c.c. of 4% glucose	3:40 P.M. 50	Dog showed no improvement (permanent) in spite of the fact that the spinal fluid sugar was maintained at a high level. Received no intravenous glucose and died at 8 P.M. in convulsions.

rapid penetration does not occur in nonhypoglycemic humans as was shown in the studies of Levinson.[5] Such rapid penetration of the meninges is probably not equaled by any other physiologic constituent.

DISCUSSION

Cases of diabetes mellitus that present unmistakable symptoms of insulin shock when the blood sugar is at a relatively high level are not unusual. Such

TABLE IV

SHOWING SPEED OF SPINAL FLUID PENETRATION FOLLOWING INTRAVENOUS GLUCOSE IN HYPOGLYCEMIC DOGS

DOG NO.	BLOOD SUGAR	SPINAL FLUID SUGAR BEFORE GLUCOSE	SPINAL FLUID SUGAR AFTER 25 C.C. 50% GLUCOSE INTRAVENOUSLY
5	61	32	78 (7 min. later)
6	34	28	73 (10 min. later)

cases usually respond to carbohydrate ingestion, despite the fact that the blood sugar values may range between 150 and 200 mg. per 100 c.c., or more. John[6] has attempted to explain such cases of shock on the basis of a decreased permeability of the corpuscles in the diabetic patient. Other observers[7, 8] on the other hand have found an increased permeability of these cells. Our own work throws no additional light on this phase of the subject. It is apparent that meningeal permeability is quite high in the ordinary case of insulin hypoglycemia, and patients in such cases recover quite promptly following intravenous glucose injections; but it is conceivable that in such cases as the one reported here meningeal permeability may be markedly decreased. Under such circumstances insulin shock might persist even though, by intravenous glucose injections, high blood sugar values are obtained. Decreased meningeal permeability could be ascertained by spinal fluid sugar determinations and, if low values are found despite high blood sugar figures, intraspinal injections of glucose might be resorted to.

CONCLUSIONS

1. Insulin, in the normal animal, has a uniform action in lowering the spinal fluid sugar. Such decreases are often of greater proportion than the blood sugar reductions.

2. There is only a temporary improvement following intraspinal glucose injections in hypoglycemic dogs. A sustained high spinal fluid sugar without a commensurate high blood sugar will not preserve the life of the animal.

3. Glucose injected intravenously during hypoglycemia enters the spinal fluid very rapidly in the experimental animal.

4. Intraspinal glucose injections should be resorted to in the hypoglycemic diabetic who does not respond to intravenous glucose injections.

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THE BLOOD LIPASE IN PATIENTS WITH PEPTIC ULCER*

Its Relation to Hepatic and Pancreatic Disease

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CHERRY and Crandall[3] found an enzyme capable of splitting olive oil in the blood of dogs after experimental damage to the pancreas. They[5] found the same lipolytic enzyme in 80 per cent of the cases of liver disease studied. In their opinion, the only conditions in which this enzyme appears in increased amount in the blood, both in the dog and in man, are pathologic changes in the liver and pancreas.

Occasional reference is made in the literature to lesions and dysfunction of the liver and pancreas associated with peptic ulcer. Thus, Gandy,[9, 10] in 1899 and again in 1920, mentioned lesions of the liver found in cases of peptic ulcer. Pathologic changes in the liver and kidneys have been suggested by LeNoir, Richet, and Jacquelin[16] as the cause of death of five patients following surgical procedures for peptic ulcer. Since the hepatic alterations could not be demonstrated by clinical methods they[18] employed laboratory tests. Unfortunately, some of the liver function tests they used were of questionable value, namely, provoked glycosuria and digestion hemoclasis. Because in a considerable percentage of cases of gastric ulcer the fasting blood sugar and the glycemic coefficient were greater than normal, LeNoir, de Fossey and Richet[15] concluded that the changes were due to disturbed hepatic function. That hepatorenal insufficiency may be found in cases of carcinoma of the stomach but is more frequent and more marked in peptic ulcer is the opinion of LeNoir, Richet and Jacquelin.[17] Vilardell and Llort[26] demonstrated by means of biopsy definite microscopic lesions of the liver in patients with peptic ulcer.

Cases of clinically manifest jaundice associated with gastric and duodenal ulcer have been reported by Tiprez and Dumont,[24, 25] and the relation of jaundice to disease of the liver and biliary tract has been discussed by Surmont,[23] Bickel,[1] and Zoepffel.[27] Masked jaundice associated with peptic ulcer and demonstrated by means of the Van den Bergh reaction has been investigated by Hadlich,[11] and by Kalk and Siebert.[14] Cholecystitis and cholelithiasis have been found accompanying ulcer of the duodenum (McVicar and Weir,[20] Judd,[13] Bruce,[2] Mix,[22] MacLaren and Oerting[19]).

Meyer[21] reported two cases of renal glycosuria with ulcer of the duodenum and thought it might be due to an associated infection of the pancreas. Jankelson and Rudy[12] stated that a nondiabetic glycosuria in cases of peptic ulcer was suggestive of an adjacent chronic pancreatitis. The relationship between lesions of the pancreas and ulcers of the duodenum and stomach has been dis-

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cussed by Ehrström and Nylander.[7] Dysfunction of the pancreas has been investigated by means of spontaneous and provoked glycosuria, hyperglycemia, and the diastase content of the blood and urine by Galli, Pecco and Polacco.[8] These authors considered pancreatic dysfunction to have been present in 32 per cent of the cases studied.

In order to apply a more reliable test to the problem of the possible association of disturbance of the functions of the liver and pancreas with ulcer of the stomach and duodenum, we have employed the method described by Cherry and Crandall[3] and have studied the lipase content of the blood of fifty patients with peptic ulcer and of twenty-five control patients.

METHOD

In each of two test tubes 1 c.c. of blood serum was added to 3 c.c. of distilled water. One tube was inactivated at 90° C. for five minutes. This inactivated tube was used as the control. To each tube were added 2 c.c. of a 50 per cent emulsion of olive oil and 0.5 c.c. N/3 sodium phosphate buffer adjusted to P_H 7.0. The contents of the tubes were thoroughly mixed and incubated at 40° C. for 24 hours. At the end of that time they were titrated with N/20 sodium hydroxide, using three drops of 1 per cent alcoholic solution of phenolphthalein as an indicator. The results have been expressed in cubic centimeters of N/20 alkali necessary to neutralize the fatty acids set free in 1 c.c. of olive oil.

In 60 determinations of inactivated serum it was found that the limits of variation were not more than 0.2 c.c. of N/20 NaOH. Since this corresponds with the results of Cherry and Crandall we have considered 0.2 c.c. as the limit of experimental error, and any reading of 0.3 c.c. or greater has been recorded as positive.

DISCUSSION

The results of the blood lipase determinations of fifty patients with peptic ulcer are found in Table I. In all cases except one roentgenologic evidences of peptic ulcer were present. The patient in case 8 was proved to have a duodenal ulcer at operation. A lipase titer of 0.3 c.c. or greater N/20 sodium hydroxide was found to be present in 38 cases, or 76 per cent. No relation between the lipase centent of the blood and the age or sex of the patient could be demonstrated.

In most cases the clinical history was the only source of information available to us by which an estimate could be made as to the approximate duration of the ulcer. An appreciation of the questionable accuracy of this method was fully realized since it is well known that many cases of peptic ulcer come to necropsy although no definite symptoms of ulcer were present during life. However, in a few cases roentgenologic evidences and previous surgical operations gave a more accurate criterion as to duration. In our studies no connection could be found between the amount of blood lipase, as indicated by the alkali titer, and the approximate duration of the ulcer.

Only four cases of gastric ulcer were studied. The blood lipase titer of these patients was 0.5, 0.6, 0.6, and 0.4 c.c. N/20 sodium hydroxide. It seems probable, therefore, that the presence of an ulcer in either the stomach or duodenum may be associated with an increase of the blood lipase titer.

The effect of alimentary lipemia by increasing the total amount of fat for the enzyme to act upon, thus liberating more fatty acids, was considered. In the control cases no alteration in the titrations due to this factor was noted. The blood was taken from the ulcer patients before or after meals or after 12

TABLE I

BLOOD LIPASE IN PEPTIC ULCER (TITER EXPRESSED IN C.C. OF N/20 NAOH)

CASE	AGE	LOCATION	DURATION	LIPASE	CASE	AGE	LOCATION	DURATION	LIPASE
1	55	G	7y	0.5	26	55	D	34y	0.4
2	44	D	5m	0.4	27	50	G	12y	0.6
3	25	GJ	?	0.6	28	42	D	9y	0.6
4	66	D	2y	1.0	29	45	D	17y	0.8
5	36	D	12y	0.6	30	35	G	5y	0.6
6	31	D	?	0.1	31	20	D	6m	0.8
7	48	D	8y	0.8	32	26	D	9y	0.2
8	34	D	?	0.1	33	45	D	12y	1.5
9	49	D	?	0.2	34	60	G	15y	0.4
10	45	D	?	1.0	35	33	D	9y	0.5
11	59	D	2m	0.6	36	53	D	10m	0.4
12	25	D	2m	0.3	37	53	D	6y	0.1
13	24	D	1m	1.4	38	51	D	4y	0.4
14	38	D	2y	0.3	39	38	D	4y	0.4
15	68	D	6y	0.3	40	34	D	10m	0.4
16	41	D	21y	0.6	41	47	D	2y	0.5
17	44	D	1y	0.3	42	40	D	6y	0.6
18	37	D	10y	0.5	43	53	D	3y	0.2
19	38	D	3y	0.6	44	46	D	8y	0.4
20	26	D	5y	0.3	45	44	D	10y	0.9
21	51	D	3y	0.1	46	30	D	6y	0.2
22	33	D	7y	0.2	47	26	D	8m	0.4
23	45	D	18y	0.5	48	36	D	3y	0.4
24	36	D	14y	0.0	49	42	D	8m	0.7
25	42	D	6m	0.2	50	61	D	6m	0.0

All were males except 24, 34, 49, and 50.

The Wassermann reaction was positive in 7 and 13 and negative or undetermined in the remainder.

Nine patients had some type of surgical procedure on the stomach, usually a gastroenterostomy.

Various types of therapy were used: alkalies, citrosalvan, mucin, or chondroitin.

hours of fasting. Alimentary lipemia either caused no change in the lipase titer, or it was so small as to be negligible.

Crandall and Cherry[4] found in 146 control patients that six gave a titration of 0.3 c.c. or more N/20 NaOH. In order to check our technic twenty-five unselected patients were studied. After making the determinations, none of the patients were found to have symptoms referable to hepatic disease or peptic ulcer. One patient was said to have "some pancreatic involvement." Three patients in this group gave positive results. The lipase titer and diagnosis in these cases were as follows: 0.5 c.c. N/20 sodium hydroxide, hypertension, nephrosclerosis and arteriosclerosis; 0.5 c.c. N/20 sodium hydroxide, possible kinking of the right ureter; 1.2 c.c. N/20 sodium hydroxide, diabetes mellitus, pyelitis, cystitis, and nephritis.

Evidence has recently been presented by Ivy, Schrager, and Morgan[28] that disturbance of hepatic function may be an etiologic factor in the occurrence of gastric and duodenal ulcer. The experiments here reported throw no light on this problem. Although we found positive evidence of disturbance of the

function of the liver or pancreas or of both together in 76 per cent of the cases of gastric and duodenal ulcer studied by a new and apparently reliable test, the lesions underlying these functional disturbances might just as well have been secondary to the ulcer as a primary and etiologic factor in its production.

CONCLUSIONS

1. An enzyme capable of splitting olive oil was found to be present in the blood of 38 (76 per cent) out of 50 patients with peptic ulcer.

2. The amount of this enzyme present, indicated by its alkali titer, had no relation to the probable duration of the ulcer or to the age or sex of the patient.

3. It is suggested that this lipolytic enzyme in the blood is due to associated dysfunction of the pancreas or liver or both.

4. This test may prove valuable in preoperative determination of the presence of one factor of the surgical risk, namely, pathologic changes in the structure or function of the liver and pancreas, in patients with peptic ulcer.

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CARCINOMA OF THE PANCREAS*

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IN AN attempt to determine the frequency and symptomatology of carcinoma of the pancreas we have reviewed 50 cases proved at autopsy, seen during the period from January 1, 1925, to January 1, 1933. The records are from the Cook County Hospital, and a smaller number are from the Research and Educational Hospital. The majority of the autopsies were performed by Dr. R. H. Jaffé, pathologist at the Cook County Hospital. Clinically diagnosed cases, even those confirmed by laparotomy but not proved by necropsy, are omitted because of the great difficulties in diagnosis. Metastatic carcinoma of the pancreas is not mentioned.

Knowledge of carcinoma of the pancreas certainly goes back to 1857, when the elder Da Costa[1] wrote of it. Mondiere[2] in 1836 is quoted as having written about it. Ancelet[3] in 1864 includes mention of it in his monograph. However Bard and Pic[4] in 1888 first described the clinical picture accepted as classical of carcinoma of the head of the gland. Knowledge of this last work has gradually pervaded the whole of medical literature. French writers since that time have accorded carcinoma of the pancreas a relatively important place in their medical literature.

The 50 cases occurring in 7,932 autopsies constitutes 0.63 per cent of all necropsies. In this group there were 942 malignancies of all sorts. Primary carcinoma of the pancreas made up 5.3 per cent of all malignancies. Carcinoma of the stomach was seen 211 times, 22.3 per cent of all malignancies. The ratio between the number of carcinomas of the stomach to those of the pancreas is roughly 4 to 1. Kolb,[5] in 1907, found 54 carcinomas of the pancreas in men among 8,777 deaths due to malignancy, and 50 in 11,266 similar cases in women. There was in his statistics a ratio of 87 carcinomas of the stomach to one carcinoma of the pancreas in male. Our figures are more in agreement

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with figures from the Massachusetts General Hospital.[6] Here in 700 necropsies, 14 carcinomas of the pancreas were found while there were only 17 cancers of the stomach. Ewing[7] from large statistics states that carcinoma of the pancreas constitutes from 1 to 2 per cent of all malignancies. Of Kauffman's[8] cases of malignancy examined postmortem, 1.76 per cent were in the pancreas.

In our series of cases males were much in the majority, as 41 were in men and only 9 in women. Correcting for the preponderance of postmortems on men, the authors find the incidence would be 41 men to 18 women. In most series described in the literature this ratio holds.

	TOTAL CASES	MEN	WOMEN	HEAD	BODY AND TAIL	ENTIRE GLAND
Mirallie[9]	104	69	35	39	7	19
Speed[10]	52	36	16			
Fuchter[11]	31	22	9			
Kiefer[12]	33	18	15	24	5	4
Wallau[13]	330	196	134	169	46	125
Ewing	354			156	40	158
Oser[14]	26			20	5	1

Though a fair share (38.4 per cent) of the autopsies are on colored people, only five of our patients were negroes.

Of the 50 in this series, in only 22 was the growth limited to the head of the gland. In 23 cases the neoplasm arose in either the body or tail. In only four cases did the neoplasm occupy the entire pancreas. One was from an aberrant pancreas in the wall of the duodenum. The high proportion of carcinoma of the body and tail, namely 46 per cent, is in striking contrast to the figures of others.

All cases in which the carcinoma arose in the common duct have been excluded. As pointed out by Shapiro and Lifvendahl,[15] who worked with practically the same material, neoplasms of the biliary passages are often mistaken for carcinoma of the head of the pancreas even at laparotomy. They showed that biliary duct carcinoma occurred much more frequently than carcinoma of the head of the pancreas.

ANATOMIC FINDINGS

Grossly the tumors of the pancreas ranged from 3 cm. in greatest diameter to as large as 12 cm. The consistency ranged from firmness to stony hardness. Characteristically their growth was invasive into the neighboring organs and the remainder of the pancreas. In no case was there any semblance of a capsule. White streaks often outlined the lymphatics through which the tumor had extended.

Microscopically it was found that 27 of the tumors were the adenocarcinoma form; 16 were scirrhus; two, medullary; two, alveolar; and one, simplex. Two are unclassified types of highly undifferentiated carcinoma. The microscopic diagnosis bore no relation to the duration of the disease or the extent of metastases. The three cases in which there were no metastases were all scirrhus.

The pancreatic ducts were often obstructed and hence distended in the distal part of the gland. The circumference of the dilated duct commonly reached 30 mm. The gallbladder was found dilated in all but two of the 19 cases of carcinoma of the head of the pancreas with jaundice, and one of these patients had had a cholecystectomy years before. Courvoisier's rule holds, therefore, in roughly 95 per cent of these cases. However in only 6 patients were the clinicians able to palpate the gallbladder.

Metastases of these tumors are very important since they dominate the clinical and pathologic picture in many patients. In only three patients were there no metastases; all three tumors were in the head of the gland and were of the scirrhus type. The lymph nodes in the upper abdomen and the liver were most regularly affected, each showing metastases in 68 per cent of the cases. Carcinomatosis peritonei was found in 22 cases, though only 9 had an ascites that was demonstrable clinically. Lymphatic spread to the mediastinal glands and the pleura was quite frequent. Even a carcinomatous lymphangitis of the right upper lobe was found in one case. Liver metastases, contrary to being small as described by Bard and Pic are of fair size averaging from 3 to 7 cm. and reaching 15 cm. in diameter.

Involvement of the stomach by direct extension of the neoplasm occurred six times, all in carcinomas of the body or tail of the gland. The duodenum was invaded seven times. This anatomic lesion will explain the common finding of blood in the stools, as well as the frequent false results of roentgenologic examination of the stomach.

Bizarre metastases are quite frequent and often occur early in the course of this disease. One carcinoma had invaded both ureters from retroperitoneal metastases and given rise to hematuria. Another had extensively riddled the submucosa of the entire small bowel. One invaded the transverse colon causing an obstruction. A fourth had caused a kink at the splenic flexure by fixing its supporting mesocolon with carcinomatous infiltration. Only one had metastases at the umbilicus. Only one had invaded the bones, the invasion being the result of direct extension from metastases in the mediastinal lymph nodes.

In contrast to the lymphatic spread which was so common, distant hematogenous metastases[16] were found in only two cases. Both showed lymphatic spread from a primary tumor of the body of the gland as well. The first such case had an extensive lymphatic spread to all the viscera of both chest and abdomen, and in addition had multiple intracutaneous nodules of carcinoma over the face, neck, and shoulders. Another case showed involvement of the retroperitoneal lymph nodes and also had an isolated metastasis in the myocardium.

PATHOGENESIS

There is no apparent connection between carcinoma of the pancreas and regeneration. Cirrhosis of the liver with associated cirrhosis of the pancreas is rarely followed by carcinoma of the pancreas. Only three such cases were found in the literature.[17] In these cases proliferation of the duct epithelium may be a precancerous lesion. In cases of chronic obstruction to the pan-

creatic ducts from any cause, the acini may atrophy while the duct system often proliferates producing buds of tissue like the islands of Langerhans. One of this series showed a moderate degree of this process. In three cases, multiple small congenital cysts were found in the pancreas, whose occurrence might suggest the theory of carcinoma arising from misplaced embryonic tissue, the cell rests of Cohnheim or the hamartomas of Albrecht. Interstitial pancreatitis as a precursor to carcinoma was not found here. Gallstones were an associated observation in only 6 of the 50 cases.

T. Cherry[18] of Melbourne produced carcinoma of the pancreas in mice, experimentally, by inducing chronic inflammation of the area by the injection of tubercle bacilli to which mice are resistant. He showed carcinoma arising from the ducts in these animals, with intermediate stages present.

Among these 7,932 necropsies, there were found two small adenomas of the islands of Landerhans. One was seen in a pancreas in which a carcinoma had arisen from the ducts. The other adenoma was purely an incidental finding at autopsy. Neither of these patients presented any symptoms of hyperinsulinism.

CLINICAL RECORDS

Since three patients entered the hospital moribund, and no adequate history could be obtained from another patient, the study is limited to 46 clinical records. Of these cases the duration of the process judged from the onset of the first symptom that could be ascribed to the lesion in question, varied from two weeks to three years for an average of 6.9 months. Only five said their trouble was of more than one year's standing.

Pain was the first symptom noted in 23 cases. Weight loss was noted first in 6, jaundice in 6, anorexia in 7, abdominal distension in 3, and edema of the legs in one. Pain and weight loss were the most constant symptoms occurring in 43 cases, or 93.4 per cent; next was anorexia in 30 cases or 65 per cent; then jaundice in 23 cases or 50 per cent; constipation in 17, or 36.9 per cent; vomiting in 16, or 34.8 per cent; and nausea alone in ten others. Diabetes was present in eight, or 17.4 per cent. Polyphagia was present in three cases. Diarrhea was a complaint of only one patient. Kiefer in his series of 33 found cachexia, including loss of weight to be the most common symptom, jaundice second, and pain third. Speed in his group also found these three symptoms to be the most common in the order named.

Pain was undoubtedly the most constant symptom. Kiefer reports it as a symptom in 21 of 33 patients. Speed has it recorded in 61 per cent of 52 cases, while Mirallie writes of it as an important symptom in 56 of 113 patients. The pain was most frequently epigastric in location, being there in roughly three-fourths of all the patients. It varied from a soreness to a most excruciating type of paroxysm requiring narcotics for relief. It occasionally radiated around the costal margins and in a few instances even up into the chest. In the case with ureteral involvement, pain radiated down to the groins. Little relation was noted between the pain and the time or type of food-taking; however, it was frequently produced at the onset by taking very large meals and fatty foods. In only two instances it was partially relieved by alkalies or by

vomiting. These two patients got relief with Sippy ulcer management for a period of two weeks, after which that was not effective.

Pain in the back occurred in association with epigastric pain in 15 cases while it was limited to the back in 2 patients. This symptom, pain in the back, has been noted in the literature particularly by Chauffard,[19] who in 1908 drew attention to its presence in carcinoma of the body of the pancreas. The pain is characteristically located in the lower dorsal and upper lumbar regions of the back, and is most often to the left of the midline. It is severe and relieved in many instances only by the *upright position of the body* as by sitting up, walking with the body bent forward at the hips, or lying curled up on the right side. Lying on the back seemed to aggravate the pain, and so quite often the patients described the pain as being worse at night or said that it first bothered them during the night. Of the 15 cases in which the pain occurred in the back, the tumor was found in 9 to involve the body or tail of the gland; while 4 were primarily in the head, and two occupied the entire organ. Eleven of the 15 patients with pain in the back showed at autopsy a carcinomatous involvement of the body of the pancreas, which suggests that this symptom may be of diagnostic significance in doubtful cases. As to the way the pain is produced, obstruction of the pancreatic duct with its subsequent distension is one theory; just as dilatation of the biliary ducts is thought to cause pain. Invasion of the celiac plexus by the newgrowth is the most commonly accepted explanation. Many cancers which have retroperitoneal metastases, as carcinoma of the stomach, liver, or lung, produce pain in the back as a distressing symptom, even in the absence of metastases to the spine. We have not observed any postural relief of the pain in any of these other tumors.

Anorexia was a major complaint of 21 cases being associated slightly more often with carcinoma of the head (56 per cent) than with carcinoma of the body of the gland (47.5 per cent). Nausea and vomiting usually followed in sequence. The loss of appetite bore no relation to the presence of icterus or to duodenal obstruction. In fact of 13 showing direct invasion of the duodenum or stomach, only 5 patients had anorexia among their chief entering complaints.

Hematemesis did not occur in any of this series even in those with malignant ulcers in the stomach although 8 of the 21 cases having gastric analyses showed occult blood in the stomach content.

Jaundice, so strikingly a part of the picture of carcinoma of the pancreas since Bard and Pic wrote of it, was by no means constantly present. It occurs here in 23 of the 50 cases. It was the initial symptom in only 6 cases. It was absent in 3 of the 22 cases having carcinoma of the head of the pancreas. Since the jaundice is due to the compression of the common duct by tumor tissue, no jaundice follows when the tumor spreads in such fashion as to miss the common duct. Jaundice was present in 3 of the 4 cases of carinoma of the entire gland, and appeared once as a result of compression of the hepatic ducts by a metastasis from a cancer in the body of the gland. Once the jaundice appeared it did not recede, save in one woman who was icteric for 4 months and recovered only to have a recurrence of the icterus 6 months later, this time with weight loss and a progressive downhill course. The icterus indices ranged up to 250.

Jaundice and pain appeared often together for of 19 cases with obstruction of the common duct due to malignancy, there was severe pain in 17 instances. Though usually in the right upper quadrant the pain varied somewhat. E.N. had his pain in the back on the left side all the time. The pain in J.B. was of ulcer type and responded to Sippy management for a time. L.S. had only a slight epigastric distress though there was a carcinoma completely obstructing the common duct and causing a very large gallbladder. J.C. alone of this icteric group was entirely free from pain. In general when present with jaundice in cancer of the head of the pancreas, the pain was usually moderately severe, aching in character, and spontaneously remittent for short periods. It bore no definite relation to postural relief save in one case. At no time had there been colics requiring morphine.

Weight loss ranged from 10 to 80 pounds, averaging 37 pounds. It was the first symptom in 7 patients and had taken place in from two weeks to one year. Kiefer described an average weight loss of 28 pounds and stressed the speed with which it took place.

Diabetes was present in eight cases, or 17.8 per cent. This is a much higher proportion of diabetes than others have given. Joslin[20] says the association is very unusual and quotes from Kiefer. Three of the patients had known of their diabetes before they entered the hospital. One of the latter first knew of her glycosuria when she consulted a physician because of epigastric pain and pruritus vulva. The blood sugar values on these 8 patients ranged from 166 to 250 mg. per cent. In 2 of these 8 cases the entire gland was affected while in 3 the tumor was in the body, and the remaining three were primarily in the head of the pancreas. In the latter three one had an atrophy of the distal portion of the gland, and another showed multiple cysts occupying the body and tail of the gland with a virtual destruction of the parenchyma.

Polyphagia was present in three cases. Two of these had diabetes to which the symptom may be ascribed. The remaining case showed no other manifestation of a suspected hypoglycemia and did not have an adenoma of the island tissue at necropsy, but instead a scirrhus carcinoma. Not a single case presented the symptom complex described by Wilder et al.[21]

Diarrhea was present in only 1 of the 46 patients. Constipation was a complaint that had been present in 17 patients for variable periods up to many years. Without a single case of fatty stools recorded this lone instance of diarrhea would indicate that loose fatty stools with jaundice occur less frequently than is supposed. The frequency of constipation only emphasizes the rarity of diarrhea.

PHYSICAL FINDINGS

On examination of the abdomen a tumor mass was palpable in 35 cases. In 25 these masses were in the right upper quadrant and had been called metastatic tumors of the liver. In 7 instances the mass was limited to the epigastrium or spread to the left hypochondrium. In 5 cases both upper quadrants showed masses. All of the masses were hard, only a few nodular. As a rule the masses in the epigastrium were fixed, but in at least two instances in

which the carcinoma protruded anteriorly from the body of the gland, a movement of 3 cm. or more with each respiration was noted.

Ascites was found in 8 cases on clinical examination. Each was due to a carcinomatosis peritonei. In one instance the carcinoma nodules could be palpated through the abdominal wall. Rectal examination in three cases revealed hard lumps in the culdesac which proved to be drop metastases. Two had been thought to be a primary carcinoma of the rectum.

Fever was present during the hospital stay of 10 patients, or 21 per cent. The temperatures ranged from 97° to 102° F. Bard and Pic thought these cases all had subnormal temperature.

ROENTGEN FINDINGS

The x-ray examination was found to be of little help in the diagnosis in most instances. On the contrary misleading information was not infrequently obtained. Of 6 cases in which fluoroscopic examinations revealed stomach defects, 4 were infiltrations of the greater curvature by the pancreatic newgrowth. Two more were reported as pyloric obstruction. In a patient having metastases to the mediastinal glands the roentgenogram was that of a carcinoma of the esophagus. A single case showed what was taken to be an extragastric mass with displacement of the stomach. One that had invaded the transverse colon gave the findings of a carcinoma of the transverse colon with obstruction. Scholz and Pfieffer[22] reported two cases of carcinoma of the tail of the pancreas showing a deformity of the greater curvature of the stomach due to an extragastric mass. On its presence one of their cases was accurately diagnosed. In carcinoma of the head of the gland a widening and stasis of the C-shaped duodenal loop has been described but was seen here only in the large tumor arising from an aberrant pancreas in the wall of the third portion of the duodenum. X-ray examination is chiefly helpful in these cases by ruling out pathologic processes in the stomach or duodenum.

Upon review of these clinical manifestations of carcinoma of the pancreas as shown by our series of proved cases, one is unable to point out anything absolutely characteristic. On the contrary the difficulties of an accurate presumptive diagnosis is impressed upon us. Carcinoma of the head of the gland affords perhaps the best opportunity of diagnosis with the symptoms of pain, icterus, and weight loss pointing the way. The jaundice is not, however, painless as has been stated, for pain was present in 17 of the 19 of this type of patient with icterus. Pain must be taken as a cardinal sign. We present the following as a typical case history of a patient with a carcinoma of the head of the gland.

CASE REPORTS

B. S., a white male, fifty-four years old, entered the Cook County Hospital July 8, 1925, and remained until September 3, 1925. He said he had been well except for a gradual loss of weight of 20 pounds in the preceding six weeks. Two weeks before entrance he had noted jaundice and marked weakness. Anorexia had appeared at the same time. During the two weeks before entrance he had taken only a little tea and cake. Pain in the right upper quadrant had been present only one week. It was dull and aching and though bothersome did not require narcotics. It did not radiate. He said that it went away if he lay quiet. He had

noticed white stools for the past two weeks. During the same time his urine was very dark. He did not have a diarrhea. Physical examination revealed a white laborer who had lost some weight but was not acutely ill. He was afebrile. The abdomen was rounded, soft and tympanitic, the liver firm and enlarged. Its edge was felt 7 cm. below the right costal arch in the nipple line. No nodules were made out in it. The rectal examination did not detect any masses. At exploration there was found ascites. The liver presented multiple metastatic nodules. There was a hard nodular mass in the region of the head of the pancreas. The gallbladder was grossly distended. The patient died in less than three months still very deeply jaundiced, and terribly emaciated. His pain had persisted throughout the course of his illness. At autopsy a carcinoma of the head of the gland was found, with complete obstruction to the pancreatic and common bile ducts. Small metastases were present in great numbers of the liver. There were small metastases in the retroperitoneal lymph nodes. The primary tumor had invaded the second portion of the duodenum and ulcerated through the mucosa.

When the tumor involves the body and tail of the gland the diagnosis is even more uncertain but in certain cases, can be presumed. In a given case where a malignancy is suspected because of the age of the patient, loss of weight, epigastric distress, and especially pain in the upper lumbar area which responds to no simple measures save perhaps a change of posture, and with no other definite source of the tumor demonstrated, even by x-ray examination, carcinoma of the body or the tail of the pancreas is to be strongly considered. In those patients having diabetes, the attention will be focused on the pancreas. Where there has been an extension of the process to the stomach, the entire picture may exactly simulate carcinoma of the stomach.

The following case is presented as typical of carcinoma of the body of the pancreas:

I. B., a colored woman, aged sixty-one years, service of Dr. E. F. Foley, entered Cook County Hospital on June 9, 1931, and remained for seventy-five days. Her entrance complaint was of an epigastric pain, which had been present for five months, and a pain in the back, which had been present for three months. She had lost about 50 pounds in weight in the preceding six months. The epigastric pain was most intense slightly to the left of the midline. It reached its height about two hours after meals, but was present all the time. She described it as an aching or gnawing pain. Neither soda nor food brought relief. Though she was not hungry and occasionally was nauseated, she did not vomit and had not induced vomiting. The pain in the back appeared two months after the onset of the epigastric pain, which had gradually spread around either costal arch to the back. The upper lumbar and lower thoracic areas were affected, particularly on the left side. This pain was at its worst when she lay on her back. Sitting up and bending forward diminished the pain. She bent forward on walking, but could stand erect for a few minutes at a time.

Physical examination showed a colored woman who had lost much flesh, but still weighed 160 pounds. Her temperature was 99.6° F. and ranged down to 97° F. in the mornings. She was not icteric. All the physical findings were in the abdomen which was rounded and soft. There was a poorly outlined hard mass in the right upper quadrant. The kidneys and spleen were not felt. An ascites was demonstrated by the presence of shifting dullness. Rectal examination showed a hard mass about walnut size fixed in the culdesac.

Her clinical course was progressively downhill. The pain and weight loss persisted. An aversion to food developed six weeks after entrance. The ascites became so marked that she was twice tapped with removal of 2,500 c.c. and again 2,000 c.c. of a clear yellow fluid with a specific gravity of 1.020. No blood was present in this fluid. The result of the laboratory study was as follows: The urine was persistently negative for albumin, sugar, or bile. The stools were negative for blood at all times. The stomach contents showed an average free acidity of seven degrees, and a total acidity of ten. The hemoglobin was 60,

the white blood count averaged 10,300, and the red count stayed close to the entrance count of 3,700,000. The blood sugar was 85 mg. per 100 c.c.; the icterus index was 4. The x-ray examination showed nonvisualization of the gallbladder after oral administration of the dye. No anomaly was found in the stomach or duodenum. On two occasions a constant deformity with constriction of the hepatic flexure of the colon was demonstrated with barium enema.

At autopsy a carcinoma measuring $8 \times 5 \times 4$ cm. was found in the body of the pancreas. There was an ascites due to carcinomatosis of the peritoneum. Numerous metastases were present in the liver. The hepatic flexure of the colon was bound to the undersurface of the liver by old adhesions.

COMMENT

It is obvious that because of the difficulties of diagnosis and the short average duration, the treatment is only palliative. The difficulties offered to surgery by the anatomic position of the pancreas and its close relation to the celiac plexus make extirpation almost hopeless, even if a diagnosis were made. As a palliative measure cholecystenterostomy has been done chiefly for relief of the icterus. A single case in this group had survived a cholecystogastrostomy. This patient was relieved of his jaundice for four months when it recurred and persisted until his death eight months postoperatively. His pain was not relieved though Speed says much relief is frequent. There is no reason to think a simple exploratory laparotomy will relieve the pain.[23]

SUMMARY

1. Primary carcinoma of the pancreas comprises 5.3 per cent of all carcinomas. It occurs about one-fourth as frequently as carcinoma of the stomach.

2. Carcinoma of the body and tail is as common as carcinoma of the head of the gland.

3. The most frequent symptoms of carcinoma of the pancreas are pain, weight loss, anorexia, jaundice, and nausea with vomiting. Diarrhea is quite rare.

4. Carcinoma of the head of the pancreas produces in three-fourths of the cases a progressively intense, moderately painful jaundice due to obstruction of the common bile duct. This cannot be differentiated from carcinoma of the biliary passages, a much more common lesion.

5. Carcinoma of the body or tail of the pancreas may be a presumptive diagnosis in the patient who is suspected of having an intraabdominal malignancy and who presents boring epigastric and upper lumbar pain partly relieved by postural changes, provided that no other primary tumor can be demonstrated. The presence of diabetes further supports this diagnosis.

6. The early occurrence of widespread metastases and the rapid progress of the disease make the treatment purely palliative.

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THE USE OF SYNTHALIN IN DIABETES MELLITUS*

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THE purpose of this experiment was to study the action of synthalin† on the glucose metabolism in a case of diabetes mellitus.

The product used was decamethylin-diguanidin, named synthalin by Frank, Nothmann and Wagner[1, 2] in Breslau and first used by them in the treatment of diabetes mellitus. It was reported to have an insulin-like action when given by mouth.

CASE REPORT

T. Q., male, aged forty-six, weighed 69 kg. This patient first developed symptoms of diabetes mellitus at the age of forty-three during an attack of pneumonia, which was followed by empyema; and at the time of the experiment he had been diabetic for three years. The diabetes had only been accurately controlled for the three weeks prior to this experiment. With a diet of carbohydrate, 76 gm.; protein, 57 gm.; fat, 183 gm.; or available glucose, "G,"‡ 127 gm.; cal., 2,179, and insulin, 20 units before breakfast and 10 units before the evening meal, the urine was free of sugar. He was an intelligent and well-trained diabetic patient.

EXPERIMENT I

Method of Study.—The diet was made up of 800 gm. of whole milk and 800 gm. of 20 per cent cream. One third of this mixture was given between

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†The preparation used in this experiment was obtained from C. F. Kahlbaum through Eli Lilly & Co., of Indianapolis, Indiana.

‡"G," or available glucose, was calculated by the formula, "G" = C +0.58P +0.10F.

TABLE I

FIRST EXPERIMENT

DATE	WEIGHT IN KG.	DIET	G	FA / G	VOLUME OF URINE	SPECIFIC GRAVITY	GRAMS OF GLUCOSE IN URINE BY FOLIN-BERGLUND	FASTING BLOOD SUGAR CONCENTRATION IN PER CENT	GRAMS OF NITROGEN IN URINE	GRAMS OF CREATININE IN URINE	REMARKS
April 22	71	C–80 P–48 F–192 Cal.–2240	127	1.5	730	1.014	0.26				Insulin 20–0–10
April 23	"	"	"	"	1170	1.010	0.77				Insulin 20–0–10
24	"	"	"	"	1790	1.012	19.07	0.197	7.8		Insulin stopped
25	"	"	"	"	1400	1.014	24.18	0.202	7.2		
26	"	"	"	"	790	1.025	17.95	0.182	6.9		Insulin 0–0–8
27	"	"	"	"	1000	1.016	8.60	0.180	10.3		Insulin 8–0–0
28	"	"	"	"	1080	1.017	11.68	0.188	8.1		Insulin 8–0–0 B. M. R. = 0%
29	"	"	"	"	1060	1.015	7.27	0.201	7.3		Insulin 8–0–0 B. M. R. = –1%
30	"	"	"	"	1320	1.010	5.28	0.192	7.3		Insulin 8–0–0 B. M. R. = –2%
May 1	"	"	"	"	1540	1.012	5.77	0.191	8.3		Insulin 8–0–0
2	"	"	"	"	1260	1.012	6.71	0.205	9.7		Insulin 8–0–0
3	"	"	"	"	690	1.016	3.77	0.174	6.1		Insulin 8–0–0
4	"	"	"	"	1060	1.010	4.24	0.190	7.8		Insulin 8–0–0 – – – Synthalin 0.0125 gm. at 8 A.M.

TABLE I—CONT'D

DATE	WEIGHT IN KG.	DIET	G	FA/G	VOLUME OF URINE	SPECIFIC GRAVITY	GRAMS OF GLUCOSE IN URINE BY FOLIN-BERGLUND	FASTING BLOOD SUGAR CONCENTRATION IN PER CENT	GRAMS OF NITROGEN IN URINE	GRAMS OF CREATININE IN URINE	REMARKS
May 5	"	"	"	"	1240	1.012	1.28	0.163	6.9		Insulin 8–0–0 – – – Synthalin 0.0125 gm. at 8 A.M.
6	"	"	"	"	1300	1.010	1.49	0.141	9.0		Insulin 8–0–0 Synthalin 0.025 gm. at 8 A.M.
7	"	"	"	"	1340	1.007	0.52	0.133	7.6		Insulin 8–0–0 Synthalin 0.025 gm. at 8 A.M.
8	"	"	"	"	1220	1.009	0.61	0.148	7.4		Insulin 8–0–0 Synthalin 0.025 gm. at 8 A.M.
9	"	"	"	"	1080	1.010	1.24	0.125	7.8		Insulin 8–0–0 Synthalin 0.025 gm. at 8 A.M.
10	"	"	"	"	900	1.013	0.88	0.095	6.9	.900	Insulin 8–0–0 Synthalin stopped
11	"	"	"	"	2050	1.008	2.05	0.161	8.7	1.102	Insulin 8–0–0
12	"	"	"	"	1700	1.006	1.57	0.130	8.4	1.259	Insulin 8–0–0
13	"	"	"	"	1080	1.014	2.82	0.135	6.1	1.059	Insulin 8–0–0
14	"	"	"	"	1360	1.012	2.86	0.148	7.6	1.183	Insulin 8–0–0
15	"	"	"	"	1290	1.011	1.09	0.154	8.1	1.240	Insulin 8–0–0
16	"	"	"	"	1400	1.012	2.24	0.145	8.4	1.077	Insulin 8–0–0
17	"	"	"	"	1210	1.016	1.69	0.167	6.2	0.864	Insulin 8–0–0

TABLE II

SECOND EXPERIMENT

DATE	WEIGHT IN KG.	DIET	"G" OR AVAILABLE GLUCOSE IN GM.	F. A. / G	VOL. OF URINE PER 24 HR. IN C.C.	SPECIFIC GRAVITY	URINARY GLUCOSE IN GM. BY FOLIN-BERGLUND	URINARY GLUCOSE IN GM. BY POLARISCOPY	FASTING BLOOD SUGAR CONCENTRATION IN PER CENT	URINARY CREATININE IN GM.	URINARY NITROGEN IN GM.	REMARKS
June 2	69.6	C–80 P–48 F–192 Cal.–2240	127	1.5	1650	1.009	11.93	13.8	0.140	1.100	6.4	
3	69.5	"	"	"	1680	1.010	10.95	11.3	0.148	0.965	5.8	
4	69.3	"	"	"	1580	1.012	16.86	17.4	0.167	1.053	6.8	
5	69.3	"	"	"	1540	1.012	17.19	16.6	0.172	0.963	6.4	
6	69.3	"	"	"	1310	1.011	12.09	12.9	0.148	1.007	6.4	
7	69.3	"	"	"	1860	1.010	11.26	11.8	0.117	0.954	7.2	
8	69.3	"	"	"	1070	1.010	13.72	13.7	0.147	0.892	6.4	
9	69.3	"	"	"	1400	1.011	12.75	12.1	0.138	0.900	7.1	
10	69.3	"	"	"	800	1.016	11.47	10.8	0.142	0.748	5.9	
11	69.3	"	"	"	1860	1.007	2.65	2.6	0.124	0.885	6.7	Synthalin 0.025 gm. at 8:00 A.M.
12	69.3	"	"	"	1790	1.005	2.77	2.7	0.133	0.716	6.1	Synthalin 0.025 gm. at 8:00 A.M.
13	69.3	"	"	"	1540	1.010	1.49	1.2	0.124	0.836	5.8	Synthalin 0.025 gm. at 8:00 A.M.

TABLE II—CONT'D

DATE	WEIGHT IN KG.	DIET	"G" OR AVAILABLE GLUCOSE IN GM.	F. A. / G	VOL. OF URINE PER 24 HR. IN C.C.	SPECIFIC GRAVITY	URINARY GLUCOSE IN GM. BY FOLIN-BERGLUND	URINARY GLUCOSE IN GM. BY POLARISCOPY	FASTING BLOOD SUGAR CONCENTRATION IN PER CENT	URINARY CREATININE IN GM.	URINARY NITROGEN IN GM.	REMARKS
June 14	69.3	"	"	"	1440	1.006	1.16	1.2	0.125	0.800	5.8	Synthalin 0.025 gm. at 8:00 A.M.
15	69.3	"	"	"	1640	1.006	1 29	1.3	0.146	0.841	7.0	Synthalin 0.025 gm. at 8:00 A.M.
16	69.3	"	"	"	1740	1.006	0.56	----	0.098	0.911	6.4	Synthalin 0.025 gm. at 8:00 A.M.
17	69.3	"	"	"	1500	1.006	0.89	----	0.121	0.858	5.9	
18	69.0	"	"	"	1620	1.011	3.66	3.7	0.148	0.853	6.3	
19	69.0	"	"	"	1000	1.014	4.62	4.5	0.104	0.909	6.1	
20	69.0	"	"	"	1120	1.011	4.00	4.1	0.102	0.896	6.0	
21	69.0	"	"	"	1800	1.004	5.19	5.4	0.112	0.720	6.8	
22	69.0	"	"	"	2000	1.007	9.59	9.3	0.127	0.876	6.3	
23	68.8	"	"	"	1500	1.008	8.65	8.8	0.128	1.020	7.0	
24	68.4	"	"	"	1840	1.009	13.19	13.2	0.123	1.011	5.4	
25	68.0	"	"	"	1660	1.009	11.02	10.0	0.149	1.137	6.0	
26	68.0	"	"	"	1200	1.009	7.25	6.9	0.121	0.987	6.5	
27	68.0	"	"	"	2200	1.005	11.75	12.5	0.120	0.965	6.7	
28	68.0	"	"	"	1380	1.013	12.39	11.6	0.148	0.953	5.6	
29	67.2	"	"	"	1100	1.017	15.70	14.4	0.143	0.974	6.1	

8 and 10 A.M., one third between 12 noon and 2:00 P.M., and one third between 6 and 8 P.M. This diet permits of carbohydrate, 80 gm.; protein, 48 gm.; fat, 192 gm.; glucose, 127 gm.; and cal., 2,240. Four analyses during the experiment indicated that the content of C, P and F of the mixture was constant.

The complete 24-hour specimen of urine was analyzed daily for glucose by the Folin-Berglund method and polariscopy, for nitrogen by the Kjeldahl process and for creatinine by the picramic acid method. The latter was used for a check on the urinary volume. Daily fasting blood sugar determinations were made by the Folin-Wu method.

RESULTS

When the patient was on the experimental diet, 20 units of insulin before the breakfast and 10 units before the evening meal were necessary to desugarize the urine. When 8 units were given before breakfast and none in the evening, the urinary glucose excretion was at a level that was desirable for study. This dosage was carried throughout the entire experiment. The average daily urinary glucose excretion during the fore-period from April 29 to May 3, inclusive, was 5.76 gm. During the synthalin period from May 4 to 9, inclusive, the average urinary glucose excretion was 1.56 gm. In the after-period, or May 10 to 17, inclusive, the average urinary glucose excretion was 1.90 gm. No toxic effect of the drug was noted.

In this experiment which was carried out the same as Experiment I, the level of glycosuria was within the desirable limits for experimentation without the use of insulin. The average of the daily urinary glucose excretion in the fore-period, June 2 to 10, inclusive, was 13.13 gm. and the nitrogen, 6.5 gm. During the period of the administration of synthalin, June 11 to 16, inclusive, the average daily urinary glucose excretion was 1.65 gm. and the nitrogen, 6.3 gm. In the first after-period, June 17 to 22, inclusive, the average urinary glucose excretion was 4.67 gm. and the nitrogen, 6.2 gm. In the second after-period, June 23 to 29, inclusive, the average daily urinary glucose excretion was 11.42 gm., and the nitrogen was 6.2 gm. No toxic effect of the drug was noted.

DISCUSSION

The daily administration of 0.025 gm. of synthalin by mouth produced a well-defined reduction in the urinary glucose excretion in this diabetic patient in both experiments. The maximum effect or complete desugarization of the urine was not reached until the fourth day of administration in the first experiment and not until the sixth day in the second experiment. The failure of the level of urinary glucose excretion to return in the after-period to that of the fore-period in the first experiment was probably due to a return of natural tolerence of the patient, brought on by the period of desugarization. This was less evident in the second experiment for the glucose excretion level in the second after-period almost reached that of the fore-period.

Because of the toxic effect on the liver reported by other investigators the use of synthalin was discontinued.

SUMMARY

The oral administration of synthalin to this diabetic patient completely desugarized the urine in four to six days. The effect was sustained over a period of several days.

The author wishes to thank Dr. R. T. Woodyatt for his valuable advice and assistance during these experiments.

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BASAL METABOLISM OF OLD PEOPLE*

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I. INTRODUCTION

IT IS not only for mere scientific interest but is important for practical purposes to determine the normal standard basal metabolism, since it will serve as the basic level when we discuss nutrition problems, such as food requirement, muscular work, and abnormal metabolism. In 1899, Magnus-Levy[1] initiated the work in this field in order to compare the metabolisms of normal and abnormal individuals.

Since then many research workers have published papers on the normal standard metabolism with improved calorimeters and technics. But the difficulty of this research work is that it requires a careful selection of subjects and technics. Especially is it true in determining the normal standard of the old age group.

In their paper Magnus-Levy and Falk presented data which showed the fact that the old age group had a lower metabolic rate than a younger group. Aub and DuBois,[2] Harris Benediet,[3] Boothby and Sandiford,[4] etc., discussed the basal metabolism of the old age group, and they are all in accord that metabolism is lower in older people, as long as they are in a normal condition.

However, it seems to me that their subjects were too few to discuss the fact fully, and in some papers the investigators did not include higher ages.

For the past several years we have carried out a series of experiments to determine reliable data on the normal standard metabolism for the Japanese. This paper is a result of such studies and concerns experimental data collected on the old age group.

II. EXPERIMENT

a. Material.—We have chosen old people of both sexes above the age of fifty years. Due to their higher age, most of them had no special work, but they enjoyed retired life. Persons having definite symptoms of illness at the

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time of study were excluded, though some of them had the so-called senile changes of mild nature occurring in the older people, such as high blood pressure, arteriosclerosis, rheumatism, and chronic nephritis. We regarded these changes to be more or less physiologic accompanying age.

The number which we have examined is 94, consisting of 44 males and 50 females. Their ages were as shown in Table I.

TABLE I

AGE	MALE	FEMALE	TOTAL CASES
50-59	13	8	21
60-69	15	16	31
70-79	10	18	28
80-89	5	8	13
90-100	1	--	1
			94

b. Method of Study.—These experiments were carried out during the spring and autumn seasons of 1929 and 1930, in order to have more uniform ranges of dry bulb temperature of 15° C. or 23° C. and dry Kata coefficient of 3.0 or 4.5, both of which were measured near the head of the subjects.

The apparatus used for the experiment was a Benedict's respiration apparatus with cot chamber. Every care was taken and the procedure carried out as the inventors[5] had advised.

III. RESULT AND DISCUSSION

We have summarized our data in Tables II and III. To determine the surface area, we used Takahira's formula[6] which was derived from actual measurements on Japanese subjects, modifying the Du Bois' height-weight formula,[7] since Japanese have a little different proportion of the body structures than do European races.

$$A = W^{0.427} \times H^{0.718} \times 74.49$$

A——Surface area in square meters
W——Weight in kilograms
74.49——Constant
H——Height in meters

a. Comparison of Different Age Groups.—A comparison of the metabolism in each age group is as follows:

Basal metabolism per square meter per hour (Table IV).
The difference between the age group of from fifty to fifty-nine and that of the eighty-year group is 3.99 calories (11 per cent) in man, and 3.65 calories (11 per cent) in woman. The decrease in each ten-year period is from 3 to 5 per cent. The decrease being gradual there is no sudden drop of basal metabolism in any age group.

Heat production per square meter per hour and age have the following correlation coefficient:

Male	-0.5173 ± 0.0745
Female	-0.3804 ± 0.0816

Though there is a little different correlation coefficient between male and female, both groups have negative correlation. Calculating with these data we found the prediction equations of heat production based on age to be as follows:

Male $H = 42.5042 - 0.1202\ A$
Female $H = 40.2902 - 0.1086\ A$

TABLE II

MALE

NO.	AGE	WEIGHT	HEIGHT	SURFACE AREA	O_2 CONSUMED PER HR.	CO_2 ELIMINATED PER HR.	R. Q.	CAL. PER HR. PER SQ. MET.	CAL. PER 24 HR.
		(KG.)	(CM.)	$(M)^2$	(L)	(L)			
1	50	49.60	154.2	1.41	11.24	8.88	0.79	38.18	1292
2	51	50.80	157.5	1.51	9.95	9.60	0.96	32.93	1193
3	54	47.00	146.0	1.34	9.71	8.33	0.86	35.31	1135
4	54	39.00	142 9	1.21	8.31	6.89	0.83	33.22	965
5	54	50.20	152.8	1.41	10.65	10.12	0.96	37.74	1277
6	55	47.10	164.5	1.51	10.48	8.75	0.83	33.59	1217
7	56	36.30	151.0	1.24	9.40	7.77	0.81	36.48	1086
8	56	49.00	158.3	1.43	10.22	9.40	0.92	35.39	1215
9	57	43 70	149.5	1.36	10.96	8.55	0.78	38.49	1256
10	58	53.10	156.8	1.53	11.59	9.85	0.85	36.84	1353
11	58	39.00	146.2	1.23	9.78	7.04	0.72	37.36	1103
12	58	47.30	142.0	1.31	9.40	8.65	0.92	35.50	1116
13	59	47.65	158.0	1.47	11.38	9.63	0.85	37.63	1217
Ave.	55.4	46.10	152.3					36.05	1187
14	60	43.00	146.5	1.33	9.47	7.39	0.78	33.40	1085
15	60	49.50	151.5	1.49	11.45	9.55	0.83	37.17	1329
16	60	40.00	144.0	1.28	9.92	8.96	0.90	38.17	1173
17	63	48.70	155.0	1.46	10.88	9.46	0.87	36.42	1276
18	63	55.20	161.0	1.57	11.02	9.16	0.83	33.54	1269
19	64	45.90	148.0	1.33	9.05	6.60	0.73	32.06	1023
20	64	41.60	149.2	1.28	9.30	6.60	0.71	34.06	1046
21	65	55.50	163.0	1.61	12.24	10.33	0.81	36.59	1414
22	65	40 25	151.5	1.33	10.07	8.37	0.83	36.69	1171
23	66	46.00	151.8	1.35	8.92	7.22	0.81	31.80	1030
24	66	52 85	164.5	1.62	12.06	9.90	0.83	36.03	1401
25	66	44 22	155 0	1.40	9.66	8.66	0.90	33.22	1116
26	66	46.90	156.0	1.46	9.96	8.07	0.81	32.83	1150
27	66	43.20	147.5	1.34	9.86	8.31	0.83	35.61	1145
28	68	42.55	155.0	1.38	10.34	8.38	0.81	36.05	1194
Ave.	64.1	46.40	153.5					34.91	1188
29	71	52.60	164.0	1.57	11.31	9.06	0.80	34.52	1303
30	72	33 65	156.0	1.26	8.11	6.19	0.76	30.11	901
31	74	37.75	147.0	1.26	9.21	7.04	0.76	34.73	1050
32	74	46.60	157 0	1.45	10.30	7.28	0.77	33.84	1178
33	75	41.60	155 0	1.34	9.74	8.69	0.91	35.88	1154
34	75	43.85	151.5	1.38	9.99	8.89	0.89	35.56	1178
35	77	40.95	139.0	1.26	8.33	6.67	0.80	31.78	960
36	78	50.00	155.5	1.48	9.58	7.74	0.78	30.91	1098
37	79	45.60	155.5	1.43	9.04	7.59	0.84	30.66	1052
38	79	50.70	159.5	1.52	10.56	8.47	0.83	33.61	1169
Ave.	75.4	44.30	154.0					33.16	1105
39	80	35.75	151 0	1.25	8.38	5.87	0.70	31.41	942
40	81	40.50	152 5	1 34	8.81	8.18	0.84	31.89	1134
41	83	48.15	167.0	1.54	9.05	7.31	0.81	28 28	1046
42	83	39.60	152.5	1 29	9.08	7.81	0.86	33.53	1062
43	89	59 30	153 0	1.54	10.67	8.43	0.79	33.18	1226
44	93	44.50	154.5	1.40	9.87	8.22	0.83	34 10	1146
Ave.	84.8	44.63	145.1					32.06	1075

This fact indicates that the basal metabolism of the human body decreases inversely to the increase of ages over fifty years old. If we consider the basal metabolism as the index of vitality, we think this decrease is a reasonable phenomenon.

TABLE III

FEMALE

NO.	AGE	WEIGHT	HEIGHT	SURFACE AREA	O_2 CONSUMED PER HR.	CO_2 ELIMINATED PER HR.	R. Q.	CAL. PER HR. PER SQ. MET.	CAL. PER 24 HR.
		(KG.)	(CM.)	$(M)^2$	(L)	(L)			
1	54	47.60	144.1	1.32	9.08	7.99	0.88	33.70	1068
2	54	41.20	141.8	1.23	7.83	6.81	0.87	31.09	918
3	55	50.30	139.5	1.32	8.41	7.65	0.91	31.43	996
4	56	42.40	144.6	1.26	8.61	7.07	0.82	32.98	997
5	57	55.00	139.6	1.37	10.51	9.46	0.90	37.77	1242
6	58	40.80	152.1	1.34	9.91	8.92	0.90	36.44	1171
7	58	33.80	149.5	1.21	9.13	8.01	0.87	36.88	1071
8	59	42.00	144.5	1.29	8.43	7.88	0.93	32.42	1004
Ave.	56.4	44.14	144.5					34.02	1058
9	60	47.30	147.5	1.39	9.51	8.15	0.86	33.33	1112
10	62	42.30	137.5	1.26	9.26	7.75	0.84	35.65	1078
11	62	41.30	140.5	1.26	7.64	6.71	0.88	29.69	898
12	62	40.90	142.0	1.23	7.98	7.26	0.91	31.96	945
13	65	50.00	136.0	1.32	9.04	8.24	0.91	33.76	1071
14	65	37.80	143.0	1.20	8.43	6.99	0.83	33.97	979
15	66	34.30	147.0	1.17	8.42	6.65	0.79	34.45	967
16	67	43.10	137.5	1.22	8.34	7.84	0.94	34.01	996
17	67	29.70	139.0	1.10	7.17	6.32	0.88	31.95	843
18	67	32.30	141.0	1.33	7.96	6.71	0.84	29.04	927
19	67	43.30	140.5	1.29	8.40	7.46	0.89	31.98	990
20	67	38.10	136.5	1.22	8.70	7.41	0.85	34.73	1017
21	67	42.90	143.0	1.29	8.89	7.53	0.85	33.51	1038
22	69	40.10	137.0	1.24	9.20	8.26	0.90	36.53	1087
23	69	42.70	148.0	1.34	8.71	7.60	0.87	31.76	1022
24	69	37.70	142.0	1.24	8.96	7.39	0.83	34.15	1016
Ave.	65.7	40.28	141.1					33.15	999
25	72	38.00	143.5	1.25	7.67	6.64	0.87	29.96	899
26	73	38.80	137.5	1.22	8.93	7.88	0.88	35.84	1050
27	73	33.50	144.0	1.19	8.07	6.98	0.87	33.11	946
28	74	40.40	141.0	1.27	9.05	7.33	0.81	34.27	1045
29	75	31.20	139.5	1.10	6.01	5.12	0.85	26.54	701
30	76	35.50	140.0	1.19	8.30	7.18	0.87	34.08	973
31	76	40.95	145.0	1.30	8.15	7.19	0.88	30.72	958
32	76	46.30	136.5	1.31	9.04	7.96	0.88	33.82	1063
33	76	40.40	150.0	1.33	9.25	7.41	0.79	29.98	957
34	76	35.15	138.5	1.15	8.45	7.35	0.87	35.91	991
35	77	46.90	146.5	1.35	8.14	6.67	0.82	29.09	942
36	77	34.70	142.0	1.18	8.31	7.15	0.86	44.33	972
37	77	40.55	146.0	1.30	8.25	6.85	0.83	30.70	958
38	77	33.20	136.5	1.13	8.73	6.52	0.75	35.74	969
39	77	39.90	145.0	1.28	9.30	7.81	0.84	35.23	1082
40	78	39.30	138.0	1.23	7.93	7.35	0.92	31.89	942
41	78	34.70	145.5	1.23	8.66	7.39	0.85	34.22	1011
42	78	35.00	141.0	1.18	6.90	6.27	0.91	28.85	817
Ave.	75.9	38.03	142.0					31.90	960
43	80	40.80	147.0	1.28	7.71	6.72	0.87	29.65	904
44	82	31.90	138.0	1.13	8.00	5.85	0.73	33.37	905
45	82	47.00	140.0	1.34	8.78	7.14	0.81	31.54	1014
46	83	37.30	148.0	1.27	7.67	6.76	0.88	29.61	902
47	83	31.70	131.0	1.08	6.95	5.04	0.73	29.61	687
48	83	32.80	127.0	1.07	6.89	6.09	0.88	31.55	810
49	84	28.95	130.5	1.03	6.08	5.08	0.83	28.55	706
50	86	30.00	138.5	1.10	6.57	5.91	0.90	29.44	777
Ave.	83.0	35.06	137.5					30.42	851

TABLE IV

AGE	MAN	WOMAN
50-59	36.05 cal.	34.02 cal.
60-69	34.91 cal.	33.15 cal.
70-79	33.16 cal.	31.90 cal.
80-	32.06 cal.	30.42 cal.

b. Sex Difference.—As Table IV shows, we noticed some difference between the two sexes. It is natural that a female requires less calories per day than a male, as the former usually is smaller in size. However, even when we compare the basal metabolism with body surface area, we notice the same differ-

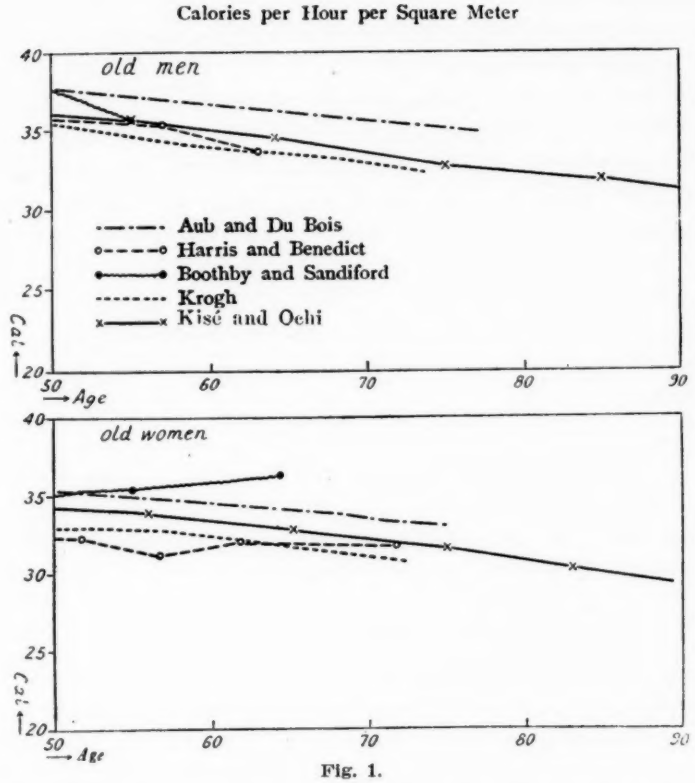

Fig. 1.

ence which is effected, since the constitution and activities of the bodies of male and female are different. In the old age group we found the average value of males to be 34.46 cal. per unit, that of females to be 32.62 cal. This difference can be seen in every age group with an average difference of 5 per cent. This value is coincident with the data reported by Magnus-Levy[1] and Benedict-Emmes.[8]

c. Comparison Between Young and Old Age Groups.—Takahira[9] first reported reliable data on the normal standard of basal metabolism of the Japanese. He dealt with the age group between twenty and fifty, and showed the averages to be as follows:

Male	37.33 ± 0.182
Female	33.84 ± 0.301

Our results in the old age groups are:

Male	34.46 ± 0.247
Female	32.62 ± 0.242

By a comparison of these two averages we find that the old age group has a lower basal metabolism by 6 per cent. The difference between the two sexes is 9 per cent among the young group, while that of the older group is 5 per cent. Here we notice that the difference of metabolism between the sexes becomes smaller with the increase of ages.

d. Comparison With Other Standards.—Aub and Du Bois[2] reported that people of from seventy-seven to eighty-three years had a basal metabolism of 35.1 cal., i.e., 10 per cent lower than that of adults. Harris and Benedict[3] reported the basal metabolism of the sixty to sixty-four group as 34.8 cal., i.e., 9 per cent lower than the young group. According to Bailey's data[10] the basal metabolism in the sixty to sixty-five year group is 5 per cent lower than that of the younger group. In the data reported by Boothby and Sandiford[11] the metabolism of a healthy man, aged sixty years, was 37.4 cal., and calculation showed that it was 10 per cent lower than that of their healthy younger group. Krogh[12] modified the Aub-Du Bois standard for ages fifteen to seventy-five and recommended a uniform reduction of 6 per cent from the standard.

We cannot compare these data with each other directly, since the groupings of the ages are different. Curves are drawn for graphic comparison. (Fig. 1.)

Our curve on male groups almost agrees with that of Harris and Benedict in the period of from fifty to fifty-nine, but in female groups it differs a little, and it lies rather near to the Aub and Du Bois' data. In general, our curve drawn with the data of the Japanese resembles that of Krogh, though our data showed less difference between the sexes.

SUMMARY

Basal metabolism was measured upon 94 normal Japanese subjects, whose ages ranged from fifty to ninety-three years. It was found that the metabolism of the aged is lower than that of younger people, and it decreases gradually without a sudden drop in any age group. The rate of decrease in each ten-year period is about 3 to 5 per cent. We found that the difference in rates between the sexes in the old age groups is smaller than in the younger groups. Comparing with the other standards, the curve drawn on our data agrees very closely with that of Krogh's modification.

The authors desire to express their appreciation to Professor Yoshio Kusama for much helpful advice and criticism throughout this work.

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THE BLOOD PICTURE IN ORAL INFECTION*

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THE data which form the basis of this report are the determination of hemoglobin percentage and the blood counts in the same cases of oral infection before and after treatment. These data were abstracted from the papers by Adams, Bryan et al., Bryant and Polevitzky, Burns, Harvey et al., Ivy, Logan, and Toren.

In the original papers only the percentages of the several kinds of leucocytes were given. From these percentages and the white blood cell count, we have calculated the absolute numbers of the several leucocyte types in the cubic millimeter.

Certain shortcomings must be pointed out in order to emphasize the tentativeness of any conclusions reached in this report and in order to point out certain precautions which should attend the collection of fresh data. The clinical condition of the patient, both general and local, should be clearly stated. The indefiniteness of our title is necessitated by the indefiniteness of several of the authors whose papers furnished the data. In the selection of cases, serious and competent effort should be made to limit the observations to patients who are suffering only from one or the other of the types of oral infection chosen for study. Usually no mention was made of the technic used to determine the hemoglobin percentage. This is necessary to reduce observations to a common term before valid comparisons can be made. We have *presumed* that the same technic was used after as before treatment. If this was done, then a comparison is legitimate, although we can form no idea of the absolute amount of hemoglobin present. The time of day at which the white blood cell count was made was generally not mentioned. The possible effects of meals or of exercise apparently were disregarded. A standard procedure is desirable, e.g., the count should be made routinely in the morning before arising and on a fasting stomach. The erythrocyte, leucocyte, and differential counts made before and after treatment in any given case should be made by the same individual, to minimize the "per-

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sonal equation.'' The differential count should be expressed not only in percentages but also in absolute numbers.

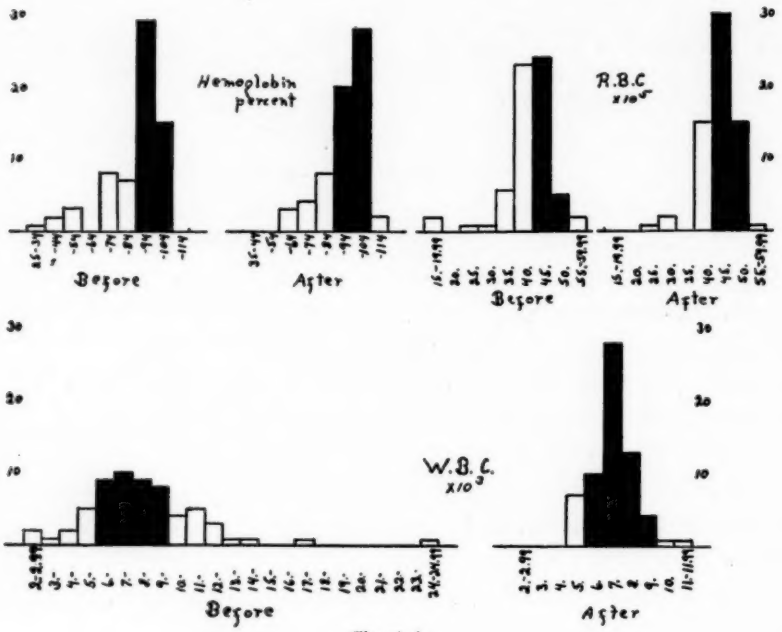

Chart 1.

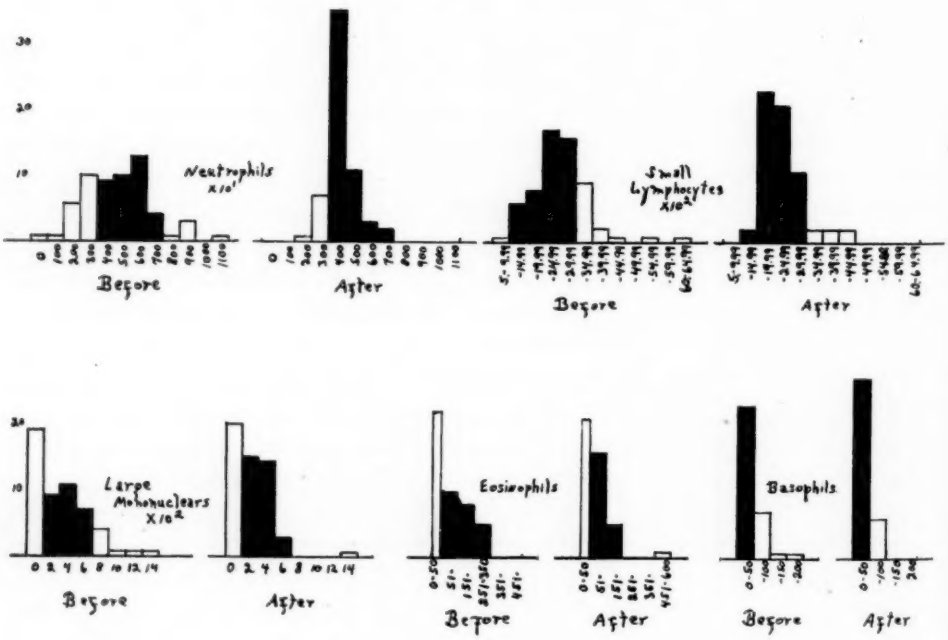

Chart 2.

The data collected are presented in Table I and Figs. 1 to 3. The cases falling within the normal ranges are represented by the blocked-in columns:

those falling outside the normal ranges, by the outlined columns. The number of cases represented by each column is indicated by the numbers at the left. Inasmuch as the number of cases used in constructing the *before treatment* graphs is the same as the number used in constructing the *after treatment* graphs, it was unnecessary to translate these numbers into percentages to permit comparisons.

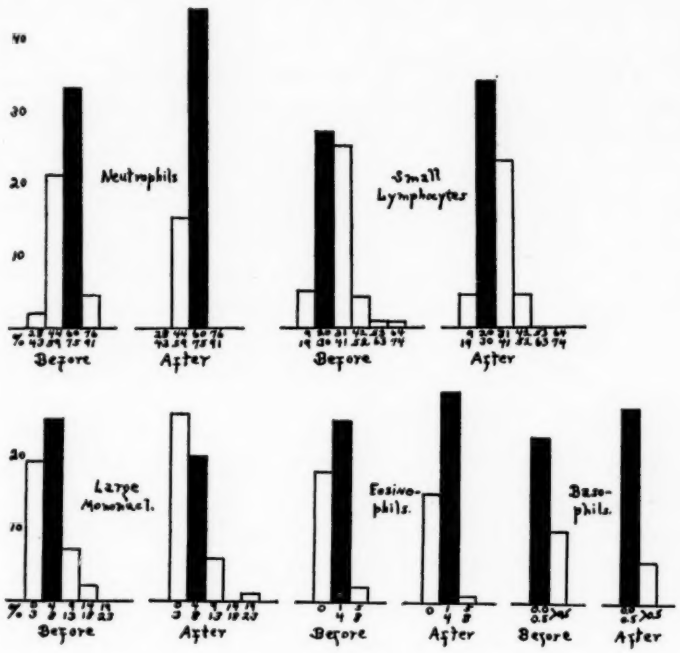

Chart 3.

CONCLUSIONS

These are subject to the limitations and criticisms mentioned at the beginning of this paper.

1. Most cases of oral infection do not induce material changes in the blood picture.

2. In some cases, however, departures from normal are seen. Some of these departures are marked. The outstanding changes, when they occur, are:

a. An anemia manifested by a lowering of both the percentage of hemoglobin and of the number of erythrocytes.

b. Either a leucopenia or a hyperleucocytosis. In the present series, the tendency toward a hyperleucocytosis is slightly the stronger.

c. The number and percentage of neutrophiles may be above or below normal. In this series, subnormal values appear the more frequently.

d. When the small lymphocytes show any change, it is usually in the direction of an increase in their number and percentage. Thus there may be both an absolute and a relative lymphocytosis.

e. The number and percentage of large mononuclears, and eosinophiles often run low.

TABLE I

	RANGE OF READINGS				NUMBER READINGS RECORDED		NUMBER READINGS IN NORMAL RANGE		% TOTAL READINGS IN NORMAL RANGE	
	BEFORE	DIFF.	AFTER	DIFF.	B.	A.	B.	A.	B.	A.
Hb. per cent	30 100	70%	58 110	52%	65	65	44	48	68	74
Total R.B.C.	1600000 5840000	4240000	2700000 5600000	2900000	64	64	29	45	45	70
Total W.B.C.	2250 24400	22150	5200 11800	6600	63	64	36	55	57	86
Neutrophiles	560 11220	10660	2940 7550	4610	59	59	36	51	61	86
Small lymphocytes	950 6460	5510	1152 4032	2880	62	63	47	57	76	90
Large mononuclears	0 1592	1592	0 1554	1554	53	53	27	32	51	60
Eosinophiles	0 348	348	0 539	539	45	43	23	21	51	49
Basophiles	0 174	174	0 98	98	33	33	23	27	70	82

f. The number and percentage of basophiles may run slightly above the normal range.

The numbers of large mononuclears, eosinophiles and basophiles actually noted in doing a "blood count" are so small that it would not be unlikely if later and more extensive studies reversed the statements listed above as e. and f.

3. Following treatment the observed values usually tend to fall within the normal range.

4. This fact supports the views: (a) that the dental infection contributed to the abnormal blood picture, and (b) that the dental treatment applied was more or less successful in combating the infection.

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4001 SPRUCE STREET

THE EFFECT OF VARIOUS COLLOIDAL AND CRYSTALLOIDAL METALLIC COMPOUNDS IN NUTRITIONAL ANEMIA OF THE RAT*

H. L. KEIL, PH.D., AND VICTOR E. NELSON, M.S., AMES, IOWA

KEIL and Nelson[1] have recently made an extensive study of the rôle of copper and certain other elements and amino acids in the regeneration of hemoglobin. This work constituted an elaboration and verification of earlier data by Hart, Steenbock, and coworkers.[2] Goerner[3] states that salts of manganese, as well as of copper, are capable of increasing the hemoglobin of anemic animals when these salts are added to salts of iron. However, he observed that colloidal solutions of manganese and copper in the presence of colloidal solutions of iron have no hematopoietic effect. Myers and Beard[4] found that higher doses of zinc and magnesium retarded blood regeneration.

The experiments recorded in this paper were instituted in order to answer certain questions: First, does manganese act like copper in hematopoiesis? Second, are colloidal Fe and Cu utilized in hemoglobin building? Third, will Fe salts injected intraperitoneally cause regeneration in anemic animals? Fourth, what effect do Zn and Mg salts have on the development of anemia? Fifth, what are the minimum amounts of Fe and Cu required for regeneration of hemoglobin? Sixth, to what extent can different compounds of Cu be utilized in hematopoiesis?

EXPERIMENTAL

All of the experiments were performed on rats. The milk used for the production of anemia was collected directly into glass containers from pure bred Holstein cows, in order to avoid contamination with copper. The salts were examined spectrographically by a Hilger quartz prism spectrograph and shown to be pure. Carbon electrodes were used in the spectrographic examination. Hemoglobin content was determined by the Newcomer method. The animals were bled by the tail.

TABLE I

DIET: WHOLE MILK COLLECTED IN GLASS

TIME IN WEEKS	AVERAGE HB. (GM. PER 100 C.C.)	PER CENT OF ORIGINAL HB.	NUMBER OF ANIMALS
0	14.6	100.0	16
2	10.6	72.6	16
4	6.2	42.4	16
6	5.0	34.2	15
8	3.8	26.0	13

It is evident from the data in Table I that the consumption of the whole milk used in these experiments produces a very marked anemia. Data have

*From the Laboratories of Physiological Chemistry, Iowa State College, Ames.
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also been obtained which show that anemia results even though iron salts are added to this milk. On the other hand, animals receiving ordinary market milk plus iron salts develop anemia much more slowly. This is due to the fact that ordinary market milk contains more copper than milk collected directly into glass. The amount of copper in market milk is variable.

TABLE II

EFFECT OF IRON AND COPPER SALTS ON ANEMIA

TIME IN WEEKS	AVERAGE HB. (GM. PER 100 C.C.)	PER CENT OF ORIGINAL HB.	NUMBER OF ANIMALS
0	3.7	100.0	12
2	9.6	259.3	12
4	13.0	351.0	12
6	15.2	410.0	12
8	14.9	402.5	12

Table II shows that copper is very potent in hematopoiesis. The animals were made anemic on whole milk, and when the hemoglobin had fallen to 3.7 gm. per 100 c.c., 0.50 mg. of Fe as $FeCl_3$ and 0.05 mg. Cu as $CuSO_4.5H_2O$ were added to the basal diet daily. When the rats were eight weeks of age the average hemoglobin was 402.5 per cent of that at the anemic level.

TABLE III

EFFECT OF IRON AND MANGANESE ON ANEMIA

TIME IN WEEKS	AVERAGE HB. (GM. PER 100 C.C.)	PER CENT OF ORIGINAL HB.	NUMBER OF ANIMALS
0	6.8	100.0	10
2	6.6	97.0	10
4	5.2	76.5	8
6	4.8	70.6	6

Table III shows that the addition of 0.50 mg. of Fe as $FeCl_3$ and 0.10 mg. Mn as $MnSO_4.4H_2O$ daily to the milk failed to stimulate regeneration of hemoglobin. Manganese therefore cannot replace copper.

TABLE IV

EFFECT OF COLLOIDAL IRON AND COPPER SULPHATE ON ANEMIA

TIME IN WEEKS	AVERAGE HB. (GM. PER 100 C.C.)	PER CENT OF ORIGINAL HB.	NUMBER OF ANIMALS
0	4.5	100.0	10
2	10.6	235.5	10
4	11.0	244.1	10
6	14.7	326.2	10
8	15.0	333.0	10

Table IV demonstrates that colloidal iron can be utilized in the building of hemoglobin. The colloidal iron was prepared from copper-free $FeCl_3$ solution. The latter solution was added to boiling Cu-free water, then dialyzed until free from dialyzable iron. After the animals had developed anemia, 0.50 mg. Fe as colloidal iron and 0.05 mg. of copper as $CuSO_4.5H_2O$ were added to the milk diet. The same results were obtained by the injection of the colloidal iron intraperitoneally.

TABLE V

EFFECT OF FERRIC CHLORIDE AND COLLOIDAL COPPER ON ANEMIA

TIME IN WEEKS	AVERAGE HB. (GM. PER 100 C.C.)	PER CENT OF ORIGINAL HB.	NUMBER OF ANIMALS
0	4.6	100.0	10
2	10.0	217.3	10
4	13.5	293.5	10
6	15.2	330.4	10

The data in Table V show that colloidal copper is also utilized in hemoglobin formation. The copper was nondialyzable. The animals received milk until they were anemic, and then 0.50 mg. of Fe as $FeCl_3$ and 0.05 mg. Cu as colloidal copper were added.

TABLE VI

EFFECT OF COLLOIDAL IRON AND COLLOIDAL COPPER ON ANEMIA

TIME IN WEEKS	AVERAGE HB. (GM. PER 100 C.C.)	PER CENT OF ORIGINAL HB.	NUMBER OF ANIMALS
0	4.0	100.0	12
2	7.8	195.0	12
4	10.6	265 0	12
6	12.0	300.0	12
8	12.9	322.5	12
10	13.8	345.0	12
12	14.6	365.0	12
14	15.1	377.5	12

Table VI shows that colloidal Fe and Cu are utilized for the construction of hemoglobin. The animals developed anemia, and then 0.50 mg. of Fe as colloidal Fe and 0.05 mg. of Cu as colloidal Cu were fed daily. The same results were obtained by injection of the iron and copper intraperitoneally.

TABLE VII

EFFECT OF INTRAPERITONEAL INJECTION OF IRON SALTS ON ANEMIA

TIME IN WEEKS	AVERAGE HB. (GM. PER 100 C.C.)	PER CENT OF ORIGINAL HB.	NUMBER OF ANIMALS
	Ferric Citrate		
0	6.2	100.0	10
2	10.0	161.4	10
4	11.4	183 9	10
6	11.6	187.1	10
8	11.0	177.5	10
10	9.5	153.2	10
	Ferric Chloride		
0	6.8	100.0	18
2	9.6	141.2	18
4	10.2	150.0	18
6	11.0	161.7	17
8	11.2	164.6	17
10	10.7	157.2	16
12	9.2	135.3	16

Table VII shows that Fe as citrate when injected intraperitoneally results in a temporary stimulation of hemoglobin formation. This experiment was performed in order to ascertain if copper was concerned in the absorption of Fe. Copper may play some part in the absorption of iron, but the data show

clearly that the rôle of Cu in hemoglobin regeneration cannot be explained on this basis alone. $FeCl_3$ acts like iron citrate although it causes necrosis. $FeCl_3$ in glycerol, however, does not have this deleterious effect when injected. The Fe as citrate and chloride was administered at a level of 0.50 mg. of Fe daily.

TABLE VIII

EFFECT OF ZINC AND MAGNESIUM SALTS ON ANEMIA

TIME IN WEEKS	AVERAGE HB. (GM. PER 100 C.C.)	PER CENT OF ORIGINAL HB.	NUMBER OF ANIMALS
	$ZnCl_2$ and $FeCl_3$		
0	12.5	100.0	6
2	11.0	87.9	6
4	9.4	75.2	6
5	8.7	69.6	6
	$MgCl_2$ and $FeCl_3$		
0	12.3	100.0	6
2	10.8	87.8	6
4	8.7	70.8	6
5	7.5	61.0	6
	$FeCl_3$		
0	13.1	100.0	6
2	11.4	87.0	6
4	9.7	74.1	6
5	8.8	67.2	6

The data in Table VIII show no effect of Zn and Mg on the development of anemia. The rate of fall of hemoglobin was the same with or without these elements. Two-tenths milligram of Zn as $ZnCl_2$ and 0.2 mg. of Mg as $MgCl_2$ were fed daily, together with 0.50 mg. Fe as $FeCl_3$. Myers and Beard[4] have

TABLE IX

EFFECT OF INTRAPERITONEAL INJECTION OF 0.002 MG. CU AS $CuSO_4.5H_2O$ DAILY

TIME IN WEEKS	AVERAGE HB. (GM. PER 100 C.C.)	PER CENT OF ORIGINAL HB.	NUMBER OF ANIMALS
0	7.4	100.0	12
2	11.8	159.5	12
4	14.0	189.2	12
6	14.8	200.0	12
8	15.0	202.7	12

TABLE X

EFFECT OF INTRAPERITONEAL INJECTION OF 0.10 MG. FE AS $FeCl_3$ DAILY

TIME IN WEEKS	AVERAGE HB. (GM. PER 100 C.C.)	PER CENT OF ORIGINAL HB.	NUMBER OF ANIMALS
0	6.0	100.0	12
2	8.9	148.3	12
4	10.4	173.3	12
6	11.8	196.6	12
8	13.2	220.0	12
10	14.3	238.3	12
12	15.1	251.6	12

stated that larger doses of Zn and Mg retard blood regeneration. We therefore believed anemia might develop more rapidly on milk plus $FeCl_3$ if these elements were included. The data show this is not the case.

Table IX shows that normal regeneration of hemoglobin is obtained by 0.002 mg. Cu as $CuSO_4.5H_2O$ injected intraperitoneally daily. The animals received milk *ad lib.* and orally 0.50 mg. of Fe as $FeCl_3$ daily. This is the smallest amount of Cu that would cause regeneration of hemoglobin to the normal level.

Table X illustrates that regeneration of hemoglobin to the normal level is obtained by injection intraperitoneally of 0.10 mg. Fe as colloidal ferric hydroxide together with 0.002 mg. Cu as $CuSO_4.5H_2O$. Smaller amounts of Fe failed to cause stimulation of hemoglobin formation to the normal level.

TABLE XI

EFFECT OF INSOLUBLE COPPER COMPOUNDS ON ANEMIA

TIME IN WEEKS	AVERAGE HB. (GM. PER 100 C.C.)	PER CENT OF ORIGINAL HB.	NUMBER OF ANIMALS
	Copper Sulphide 0.02 Mg. Cu		
0	6.0	100.0	6
2	11.0	183.3	6
4	12.3	205.0	6
6	11.5	191.6	6
8	11.7	195.0	6
10	12.0	200.0	6
12	13.4	223.3	6
14	14.8	246.6	6
	Copper Hydroxide 0.005 Mg. Cu		
0	6.1	100.0	6
2	11.2	183.5	6
4	12.0	196.7	6
6	12.8	210.0	6
8	14.1	231.2	6
10	13.8	226.3	6
12	14.2	232.8	6
14	15.0	246.0	6
	Cuprous Oxide 0.01 Mg. Cu		
0	4.2	100.0	6
2	8.8	209.5	6
4	11.9	283.5	6
6	13.6	323.8	6
8	14.6	347.0	6
9	14.8	352.0	6
	Cuprous Iodide 0.01 Mg. Cu		
0	5.0	100.0	6
2	9.4	188.0	6
4	11.3	226.0	6
6	13.5	270.0	6
8	14.5	290.0	6
9	15.1	302.0	6

The data in Table XI show that the animal body can utilize various copper compounds in the production of hemoglobin, even though these salts be very insoluble. The character of the anion apparently has no effect on the utilization of the copper.

SUMMARY

1. Manganese cannot replace Cu in the synthesis of hemoglobin.
2. Colloidal Fe and Cu are utilized in hematopoiesis.
3. Although the intraperitoneal injection of Fe salts into anemic animals

causes a temporary rise in hemoglobin, it is evident that the main function of Cu is not in the absorption of iron.

4. Zinc and Mg have no effect in the development of anemia.

5. Two-thousandths (0.002) mg. Cu as $CuSO_4.5H_2O$ and 0.10 mg. Fe as colloidal $Fe(OH)_3$ are the minimum amounts of these elements that will cause a regeneration of the hemoglobin to a normal level in the anemic rat.

6. Cu_2O, CuS, $Cu(OH)_2$, and CuI are readily utilized by the anemic rat in hemoglobin building. The sulphide is less efficient than the other salts employed.

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EXPERIMENTS IN THE USE OF A MINERAL WATER IN THE MANAGEMENT OF DIABETES MELLITUS*

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THE purpose of these experiments was to study the effect of a mineral water when used for long periods in the management of diabetes mellitus. Recently companies bottling many of the highly advertised mineral waters have made extravagant claims for their water of beneficial effects in diabetes mellitus. With this in view the following investigation was undertaken to determine whether or not there was any demonstrable beneficial effect derived from the use of one of these waters.

CASE REPORTS

CASE 1.—R. P., male, aged thirty-nine years, first had symptoms of diabetes mellitus at the age of twenty-nine. The disease had run a rapid course, and he had, to the date of the experiments, lived on a quantitative diet with insulin for eight years. He was an intelligent, trained, and experienced patient. His usual diet contained carbohydrate, 105 gm.; protein, 127 gm.; and fat, 183 gm. With this diet he remained in good health but not free of morning glycosuria on an insulin dosage of 75 units.

CASE 2.—H. E., male, aged fifty years, had become diabetic at the age of forty and had required insulin treatment for seven years. This patient was also a very intelligent, trained, and experienced diabetic patient. His customary diet contained carbohydrate, 94 gm.; protein, 74 gm.; fat, 181 gm.; and cal., 2,301, estimated "G,"† or available glucose, 155 gm.; on this diet the urine was usually sugar-free with an insulin dosage of 50 units, divided into 40 in the morning and 10 in the evening.

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†The "G," or available glucose, was calculated by the formula, "G" = C + .58P + .10F.

CASE 3.—W. S., male, aged thirty-two years, had first developed symptoms of diabetes mellitus in 1919 at the age of twenty. He first had insulin management in 1923 and went into coma in 1924 at the age of twenty-five. He had been, for seven years, at the time of these experiments on a diet of "G," 175 gm.; cal., 2,275, and insulin, 35 units before breakfast and 16 units before the evening meal. He had remained in good health but not free of glycosuria in the morning and was also an intelligent, well-trained and experienced patient.

CASE 4.—H. D., male, aged forty-five years, had first become diabetic in 1920 at age of thirty-four. By very accurate quantitative management of the diet, he was kept alive until the advent of insulin in 1922 at which time this therapy was started. For nine years and at the time of these experiments, he had been taking 50 units of insulin per day, and he had been in good health but not free of morning glycosuria. His usual diet was "G," 180 gm.; cal., 2,250. He was also a well-trained, intelligent, and experienced patient.

CASE 5.—H. A., male, aged fifty-eight years, first developed diabetes mellitus at age of forty-five. In 1923, at age of fifty years, he was placed on a diet of carbohydrate, 100 gm.; protein, 67 gm.; fat, 156 gm.; "G," 155 gm.; and cal., 2,072, and started on insulin, 32 units before breakfast and 16 units before the evening meal. At the time of these experiments he had been on this program for eight years, had remained in good health; the urine usually was free of sugar. He also was a well-trained, an intelligent, and experienced patient.

All of these patients had been continuously under the observation of Dr. R. T. Woodyatt.

The Mineral Water.—The mineral spring water used in these experiments was crystal clear and, according to the analysis submitted by the bottler, contained 120 parts per million of solid material. The distribution of the identified anions and cations was reported as follows:

H_2CO_3	as	$CaCO_3$	=	38.00	parts per million
Chlorides	as	Cl	=	8.00	parts per million
Sulphates	as	SO_4	=	18.55	parts per million
Silicon	as	SiO_2	=	8.25	parts per million
Aluminum	as	Al_2O_3	=	3.52	parts per million
Iron	as	Fe_2O_3	=	3.58	parts per million
Calcium	as	Ca	=	4.46	parts per million
Magnesium	as	Mg	=	1.13	parts per million
Sodium and Potassium	as	Na	=	24.3	parts per million
Manganese	as	Mn	=	0.08	parts per million
Ammonia	as	NH_3	=	0.04	parts per million
Nitrates	as	NO_3	=	0.02	parts per million

The radioactivity of the emanation, when in equilibrium with its parent, from one gallon of this water was reported to be in the neighborhood of 1.5×10^{-10} millicuries.

EXPERIMENTS

Methods of Study.—Each patient was hospitalized during the periods of study and placed on a diet and dosage of insulin that would permit a slight glycosuria during the entire 24 hours without acidosis. The same insulin preparation was used during all of the experiments. Two control periods were run on each patient with a week intervening. At the end of the second period the patient was discharged from the hospital using his usual diet, dose of insulin, and drinking one gallon of the mineral water each day. After using the water from one to seven months he was again hospitalized, placed on the

diet and dosage of insulin used in the control periods, the water continued, and the urinary glucose determined.

The glucose in the urine was determined by the Folin-Berglund method and polariscopy, the nitrogen by the Kjeldahl method and the creatinine by the picramic acid method. The latter was used for a check on the volume of urine.

Experiment 1.—Case of R. P., weight 73 kg. A diet of carbohydrate, 87 gm.; protein, 76 gm.; fat, 199 gm.; "G," 151 gm.; and cal., 2,443, with insulin, 32 units before breakfast, 16 units before the evening meal and 4 units at 3 A.M., permitted a glycosuria without acidosis. The average daily urinary glucose excretion during the first control period was 14.43 gm., and the urinary nitrogen, 11.1 gm. In the second control period the average daily urinary glucose excretion was 13.54 gm., and the urinary nitrogen was 12.8 gm. After he had drunk one gallon of the water per day for seven months, the average daily urinary glucose excretion was 15.76 gm., and the urinary nitrogen was 14.7 gm.

Experiment 2.—Case of H. E., weight 60 kg. A diet of carbohydrate, 92 gm.; protein, 54 gm.; fat, 172 gm.; "G," 141 gm.; and cal., 2,132, and twenty-four units of insulin before breakfast and 12 units before the evening meal permitted a continuous glycosuria without acidosis. The average daily urinary glucose during the first control period was 8.61 gm., and the nitrogen, 8.8 gm. In the second control period the average daily urinary glucose was 11.98 gm., and the nitrogen, 10.1 gm. After using one gallon of the water per day for one month, the glucose was 13.14 gm. and the nitrogen, 8.8 gm. After using the water for four months, the average daily urinary glucose was 12.50 gm. and the nitrogen was 10.1 gm. After using the water for seven months, the average daily urinary glucose excretion was 10.34 gm. and the nitrogen, 11.6 gm. In this experiment another control period was run after the water had been discontinued for one month. The urinary glucose then was 7.94 gm. and the nitrogen, 9.8 gm.

Experiment 3.—Case of W. S., weight 66 kg. A diet of carbohydrate, 92 gm.; protein, 79 gm.; fat, 187 gm.; "G," 156 gm.; cal., 2,367; and insulin, 28 units before breakast and 12 units before the evening meal, permitted a slight continuous glycosuria without acidosis. The average daily urinary glucose excretion in the first control period was 9.11 gm. and the nitrogen, 10.5 gm. In the second control period the glucose excretion was 10.01 gm. and the nitrogen, 11.5 gm. After using one gallon of the water per day for three months, the average daily urinary glucose excretion was 8.51 gm. and the nitrogen, 12.4 gm. After using the water for six months there had been no reduction in the daily insulin requirements, and the experiment was discontinued without another period of study in the hospital.

Experiment 4.—Case of H. D., weight 65 kg. A diet of carbohydrate, 92 gm.; protein, 79 gm.; fat, 187 gm.; "G," 156 gm.; cal., 2,367; and insulin, 24 units before breakfast and 16 units before the evening meal, permitted a slight continuous glycosuria without acidosis. The average daily urinary glucose excretion during the first control period was 6.84 gm. and the nitrogen, 11.6

gm. During the second control period the glucose was 7.17 gm. and the nitrogen, 12.7 gm. After drinking one gallon of the water per day for three and one-half months, there had been no reduction in the daily insulin requirements, and the experiment was discontinued without another period of study in the hospital.

Experiment 5.—Case of H. A., weight 65 kg. A diet of carbohydrate, 92 gm.; protein, 79 gm.; fat, 187 gm.; "G," 156 gm.; cal., 2,367, and insulin, 32 units before breakast and 16 units before the evening meal, permitted a continuous glycosuria without acidosis. The average daily urinary glucose excretion during the first control period was 12.99 gm. and the nitrogen, 14.7 gm. During the second control period the glucose excretion was 8.17 gm. and the nitrogen, 14.7 gm. After drinking one gallon of the water per day for seven months, there had been no reduction in the daily insulin requirements, and the experiment was discontinued without further study in the hospital.

SUMMARY

A study of the effect on diabetic patients of their drinking one gallon of mineral water per day for periods of from one to seven months was made. No beneficial effects could be demonstrated.

The author wishes to thank Dr. R. T. Woodyatt for his valuable advice and assistance during this study.

THE DYNAMIC CONSEQUENCES OF AN AURICULOPERICARDIAL FISTULA*

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IT IS generally known that pericardial tamponade has a deleterious action on the circulation, and when acute and severe enough may lead to death. Animal experiments, particularly those of Katz and Gauchat,[1] have shown that the action of pericardial tamponade is primarily to interfere with the flow of blood into the intrapericardial veins. Intrapericardial pressures in excess of 300 mm. of oil (saline) lead to circulatory death. It is the fear of establishing fistula with the resulting tamponade effects that has acted as a deterant to pericardial and cardiac punctures. However, there seems to be an impression that a communication between the pericardium and an auricle is less serious than a communication between the pericardium and a ventricle, the aorta or the pulmonary artery.

In the present investigation an attempt was made to determine the dynamic consequences of producing an auriculopericardial fistula, and in particular to see what risk, if any, was involved in such a procedure.

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METHOD

Six dogs, anesthetized with morphine and barbital, were used in this investigation. Artificial respiration was introduced and the chest opened. Chicago blue (15 mg. per kg.) was injected intravenously to render the blood noncoagulable. A pericardial cannula was inserted and connected with a saline manometer. The carotid artery, jugular vein, and a pulmonary vein branch were isolated and cannulated. The first was connected with a mercury U-tube manometer and the other two with saline manometers. After a control record was obtained on a smoked paper kymograph a small opening was made in the pericardium through which a rent about 5 mm. in length was made in the tip of the right or left auricle and the pericardial opening quickly closed. The changes in pressure during and following this procedure were recorded on the kymograph and the data analyzed. As a control 50 c.c. of blood were removed from an animal and quickly injected into the pericardium, and its effects studied.

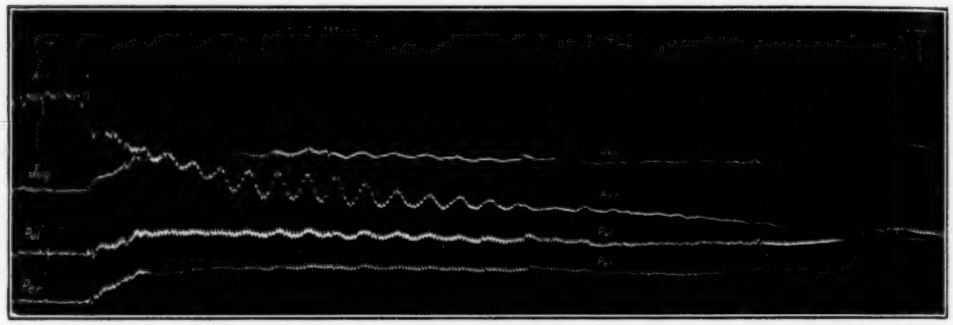

Fig. 1.—A graph showing the typical effect of an auriculopericardial fistula. *Art*, is the mean arterial pressure; *Jug.*, *Pul.*, and *Per.*, represent the mean pressure, respectively, in the jugular vein, pulmonary vein, and pericardial sac. Time in five-second intervals. Arrow indicates time of establishment of auriculopericardial fistula. Note the Traube-Hering waves and the homologues in the venous and pericardial pressure curves.

RESULTS

The results are summarized in Table I and a typical experiment shown in Fig. 1. After the establishment of the auriculopericardial fistula, there was an immediate drop in the mean arterial blood pressure and a corresponding rise in the mean pressures in the jugular vein, pulmonary vein, and in the pericardial sac, following which the pressures tended to level off but continued to change gradually in the original directions until death ensued. The mean arterial pressure just before death approximated 5 to 15 mm. of mercury. At this time the pulmonic venous pressure was found to exceed the jugular pressure in all but one experiment, regardless of whether the fistula was in the right or left auricle. The intrapericardial pressure in no instance rose above 140 mm. of saline following the establishment of the fistula, a figure which is about one-half of that found necessary to cause death with experimental pericardial effusion (Katz and Gauchat[1]). In some experiments the intrapericardial pressure was higher than the intravenous pressure; in others it was lower.

DISCUSSION

The lack of correspondence of the intrapericardial pressure with the intravenous pressures is in part due to the inability to establish absolutely the correct zero pericardial pressure level. In addition, the cyclic heart action affects the pressure in the pericardial sac in an opposite manner to the extrapericardial venous pressures. For example, ventricular systole lowers the intrapericardial pressure, but raises the venous pressures. In some experiments the last factor may explain the presence of an intrapericardial pressure lower than the venous pressures. The presence of an intrapericardial pressure higher than the venous pressures in other experiments is more difficult to explain. It is conceivable, however, that with a rent in the auricle this part of the wall of the heart may act as a one-way flapper valve, preventing a flow back from the pericardial sac. In this way the pericardial sac might be considered as acting as a maximum pressure manometer.

TABLE I

EXPER.	MEAN ARTERIAL BLOOD PRESSURE		MEAN PRESSURE IN JUGULAR VEIN		MEAN PRESSURE IN PULMONARY VEIN		MEAN PRESSURE IN PERICARDIAL SAC BEFORE DEATH	WHICH AURICLE CUT	TIME OF DEATH AFTER AURICLE CUT
	CONTROL	BEFORE DEATH	CONTROL	BEFORE DEATH	CONTROL	BEFORE DEATH			
	mm Hg	mm Hg	mm.H_2O	mm.H_2O	mm.H_2O	mm.H_2O	mm.H_2O		min.
1	145	5	25	90	65	110	140	right	13½
2	135	5	–	–	60	120	100	left	8
3	55	5	35	90	50	90	140	right	8½
4	60	5	35	50	60	60	55	right	8½
5	90	5	30	90	60	80	70	left	16
6	105	15	60	135	100	140	120	right	12

Tamponade per se cannot be responsible for the serious dynamic consequences of auriculopericardial fistula. Previous workers[1] have found that the pressure in the pericardium causing death in tamponade per se is decidedly greater than the pressure found in the pericardium at the time of death in our animals. This difference cannot be due to the loss of blood, which never exceeded 25 or 30 c.c. in our experiments. Furthermore, the removal of 50 c.c. of blood from the circulation and its rapid injection into the pericardial sac had no appreciable dynamic effect.

The other factor operating in auriculopericardial fistula appears to be the action of the fistula itself, and the following explanation is offered: When the fistula is made, the pressure in the auricle and pericardial sac tend to be equalized, the intrapericardial pressure rises and thereby increases the resistance to onward flow of blood from the extrapericardial veins. This in turn results in a damming up of blood in the extrapericardial veins with a consequent rise in their pressure. The rise in pressure, however, soon reflects itself in a similar elevation in the intrapericardial veins, auricle, and then in the pericardial sac itself. Thus a vicious cycle is started. There is no chance for

[1]Katz, L. N., and Gauchat, H. W.: Observations on Pulsus Paradoxus (With Special Reference to Pericardial Effusions). II. Experimental, Arch. Int. Med. 33 371, 1924.

a gradient of pressure to be established between the intrapericardial veins and auricles (and the diastolic pressure in the ventricles) and the pericardial sac, which is essential to prevent stagnation of the circulation and death.

These experiments show that a persistent opening between an auricle and the pericardial sac is associated with danger, even though the amount of bleeding into the pericardial sac be small.

SUMMARY

The dynamic effects of a persistent auriculopericardial fistula experimentally produced were studied. It was found that the fistula resulted in a serious handicap to the normal circulation and quickly led to death. This effect is apparently not due entirely to a tamponade action per se but to a decrease in the pressure gradient between the extrapericardial veins and the ventricle during ventricular filling produced by the presence of the fistula.

We wish to thank Dr. Louis N. Katz, at whose suggestion this study was undertaken, for his advice and criticism.

A TYPHOID-LIKE INFECTION ASSOCIATED WITH AN ORGANISM RESEMBLING *BACILLUS PROTEUS PSEUDOVALERIAE* (DE ASSIS)*

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THE case here reported is one of infection with an organism closely resembling *Bacillus proteus pseudovaleriei* (de Assis).

The patient was a man of Canadian birth, a salesman by occupation, aged fifty-eight. The family and history were of no special interest except for the fact that four years prior to the present illness the patient developed hematuria and was operated upon for a papillomatous growth of the bladder. The growth was removed by fulguration in a one-stage operation, and radium seeds were implanted in the base of the growth. Recovery was uneventful, but several months later the patient developed difficulty in urination and was able to urinate only while lying down. It became necessary to remove a stone which had formed around one of the radium seeds and which acted as a ball valve. This procedure was accompanied by severe cystoscopic shock and rigors. During the time that the patient suffered from urinary symptoms prior to operation, he had a considerable number of mild attacks of precordial pain on exertion that had been diagnosed clinically as well as by electrocardiogram as of coronary origin. These symptoms ceased after the operation.

The present illness followed a late lunch which consisted of a spiced beef sandwich. At the time this was eaten the patient thought it had a peculiar taste. He experienced a sense of epigastric distress that became worse the next day. However, on the following day he dined in a speakeasy and had two gin cocktails before dinner. The second day he had a sense of dull epigastric ache. On the third day he had severe chills lasting several hours followed by fever and sweating. During the next two weeks the symptoms became more severe and the chills and fever more frequent. There was no regular interval between the attacks.

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Anorexia and prostration were marked, and the patient lost weight rapidly and entered into a typhoid state. At this point he was admitted to the Fourth Medical Service at Bellevue Hospital. At the time of admission abdominal pain on the right side was the predominant symptom. Examination of the abdomen was negative.

Shortly after admission the patient became jaundiced. The van den Bergh test was positive and the stools were clay colored. There was rigidity in the right upper quadrant and some tenderness in the region of the gallbladder. The blood chemistry was negative. The patient began to have steady hiccoughing, and the urine was found to contain pus. Several infusions of glucose were given intravenously. The blood chemistry now showed nonprotein nitrogen 75, sugar 120, and creatinine 2.5. Blood cultures taken shortly after admission were positive and a gram-negative bacillus was found in the blood. The Widal reaction was negative. The same organism was isolated from the feces. The temperature curve gradually subsided and after a stay of three months in the hospital the patient went to his home. Following his return to New York in October he complained of severe pains in the muscles of the upper thorax. These pains were so severe as to require the use of a sedative. The x-ray of the chest and spine were negative, and the patient was given a series of heat and ultraviolet ray treatments followed by complete cessation of the pains. At the present time he is in good health and has regained weight and strength.

During bacteriologic studies of blood cultures from this patient, we isolated a bacillus which appears to be closely similar to the *Proteus pseudovaleriei* isolated by A. de Assis in Brazil in 1926.[1]

Blood cultures were made in vitamin broth and nutrient agar pour plates. The first culture, taken April 29, 1932, the day after admission, showed a gram-negative motile bacillus in both broth and plates after twenty-four hours. Another culture was taken May 7, eight days later, which was also positive. A third culture, taken May 26, was sterile.

Agglutination tests: Normal saline suspensions of the growth on an eighteen-hour agar slant and an eighteen-hour broth culture were tested with dilutions of 1:50 and 1:100 specific typhoid and paratyphoid A and B sera. No agglutination occurred.

Repeated Widal tests of the patient's serum proved negative with typhoid and Para A and Para B.

On May 26, nineteen days after this organism had been recovered from the patient's blood, the patient's serum was tested with the organism. Serum agglutinated through 1:2,500, negative 1:5,000. On repeating the test complete agglutination was found in 1:3,200, negative 1:5,000. Tested with typhoid Para A and Para B the serum again proved negative.

Stool culture of June 16, seventeen days after defervescence, on Endo plates showed a single colony which resembled morphologically and culturally, the organism isolated from the blood, and agglutinated with the patient's serum of May 26 through 1:3,200, negative 1:5,000.

These results showed that the organisms isolated from the blood and from the stool were identical, and the fact that it agglutinated with the patient's serum in a dilution of 1:3,200 showed that it was the cause of the infection.

In 1910, Rottkay[2] described a fatal case of typhoid-like disease from which he was able to recover an organism related to *B. Proteus vulgaris* (Hauser) which he isolated from the feces and after death from the spleen and ileum.

Boycott[3] in 1906 isolated from the stool of a case of atypical typhoid, two strains of Valerei which did not agglutinate with the patient's serum. This patient's serum was agglutinated with Para B. although no paratyphoid organisms were isolated from the stools.

De Assis in 1926 recovered an organism from the blood of a case of typhoid-like infection on two occasions, that strongly agglutinated with the patient's serum, but did not agglutinate with typhoid, Para A and Para B. He called his organism *Proteus pseudovaleriei.*

DESCRIPTION

Morphology: a gram-negative actively motile bacillus of varied size, from coccoid to the longer threadlike forms, morphologically resembling the typhoid bacillus.

Subcultures were made on the following media:

a. Nutrient agar slant showed a moist spreading grayish white fluorescent growth.

b. Broth showed a rapid growth, of even turbidity, with the formation of a collar or ring at the surface, and sediment after forty-eight hours. No pellicle was formed.

c. Blood pour plates showed greenish colonies similar to typhoid.

d. Endo plates showed bluish transparent colonies similar to typhoid. Broth cultures kept in the ice box a week or more and subcultured on endo showed presence of "phage plaques."

e. Triple sugar agar slants were inoculated with colonies from Endo plates and showed acid "butt" with gas production and a colorless slant similar to the paratyphoid-enteriditis group.

f. Litmus milk showed acid in twenty-four hours but was not clotted. Later it became decolorized, with partial coagulation. No digestion or peptonization.

These cultures were transplanted to various carbohydrate fermentation broth tubes containing Andrade as the indicator. Dextrose, levulose, maltose, mannite, saccharose, xylose, salicin, arabinose, raffinose, and glycerol were fermented with gas production. Lactose was not touched until after seven days, then it gave a faint acid reaction. Dextrin showed slight reaction after seventy-two hours. No gas was produced in either lactose or dextrin. Inulin and dulcite were not touched. These fermentation tests were repeated twice and all three reactions on the same lot of media proved the same. From 50 per cent to 100 per cent gas was formed.

PATHOGENICITY

We inoculated two 250 gm. guinea pigs intraperitoneally with 0.5 and 0.75 c.c. of eighteen-hour-broth culture. After three hours the pigs became quiet and drowsy, did not eat, and the hair looked roughened. Eighteen hours later they showed signs of prostration, had not eaten any food, and when placed on their backs were barely able to right themselves. Within twenty-four hours both pigs were dead. The same organism was recovered in pure culture from the peritoneum and heart's blood of both pigs.

There was a considerable amount of injection at the site of the inoculation. The peritoneum contained about 10 c.c. of mucoid fluid. The mesentery and omentum were injected. The suprarenal glands were hemorrhagic. The spleen of one pig was slightly enlarged, the gallbladder was quite distended.

DISCUSSION

The biologic characteristics of our organism places it in the genus Proteus. In 1906 Boycott described a bacillus (valeriei 21) which our organism closely

resembles in motility and fermentation, although our bacillus does not ferment dextrin and dulcite; while his does not ferment salicin, and does not touch lactose at all. De Assis, in 1926, described a bacillus which our bacillus resembles, except that ours ferments saccharose, raffinose, and glycerol, while his did not. Our bacillus resembles Castellani's *Proteus asiaticus* in fermentation, but differs in its reaction on litmus milk, and in motility. Our strain resembles Hauser's vulgaris in its reaction on saccharose, dextrin, dulcite, and glycerol, but it does not resemble it in its reaction on arabinose, salicin, raffinose, mannite, or litmus milk. The difference in the reaction on dextrin may be due to the variation of dextrin.

Our organism resembles the *Proteus pseudovaleriei* of de Assis in its fermentation reactions more closely than it does Hauser's Vulgaris or the Valerei of Castellani or of Boycott, and by the fact that his patient, like ours, recovered, while all of the others died.

SUMMARY

An organism isolated from two different samples of blood and from the stool of a patient with a typhoid-like infection, strongly agglutinated with the patient's serum but did not agglutinate with typhoid, Para A or Para B. This organism may be classed with that of de Assis.

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LABORATORY METHODS

AN IMPROVISED BACTERIOLOGIC INCUBATOR*

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A HIGH-PRICED bacteriologic incubator costing from $100.00 to $300.00 can be improvised at the very moderate cost of $20.00 or less with no sacrifice in the quantity or quality of the results to be obtained from its use. The improvised incubator can be assembled in less than fifteen minutes with only four articles that can be easily and quickly obtained, especially for those who live in a city.

A small, used refrigerator made to hold 25 pounds of ice makes a very satisfactory sized cabinet for an incubator large enough to do the bacteriologic work of a moderately large hospital. I assembled an excellent incubator, using a top icer of the above size which was purchased from a storage company for $1.50. The refrigerator was as good as new and the factory finish on it was still good.

The second article needed for the construction of the incubator is an electric heating element and regulator or electric thermostat. This can be purchased from any good laboratory supply company for not more than $16.00 and large enough to keep the entire inside of the refrigerator at 37° C. or body heat.

The third article needed to make the first class incubator is an accurate thermometer with which to regulate the electric thermostat and check the temperature of the incubator when in use. The thermometer can be obtained from the same supply company as the thermostat for a dollar or less or a suitable one can be obtained at a drug store.

The fourth article needed is a one-holed stopper which can be obtained from the supply company.

With these articles at hand one is ready to begin assembling the incubator. First open the door to the refrigerator and remove the tube connecting the ice compartment with the storage compartment by raising up and pulling out on the bottom of the tube. This leaves an opening down through the bottom of the refrigerator. Next, the thermostat is placed on the lower shelf in the left rear corner and the electric cord will just be in position to go down through the hole in the lower shelf, but first the electric connection will need to be temporarily removed as the hole in the floor of the refrigerator is not large enough to pass the connection through. After the cord has been

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put through the floor, the electric connection can be replaced and made ready to plug into a wall socket supplying 110 volt current.

If a laboratory type of thermometer is to be used, an inch hole should be drilled through the top door and a cork with a hole made through it the size of the thermometer can be inserted in the hole. The thermometer is then suspended with the bulb well within the refrigerator by passing it through the stopper so that the mark for body heat or 98.6° F. is well above the outside of the cabinet. Any accurate thermometer may be used by placing it on the shelf inside the refrigerator and the readings taken quickly upon opening the door or before the thermometer has time to change enough to be of any importance. However, for greater accuracy the laboratory thermometer through the pierced wall is recommended, so that the temperature can be read without opening the door.

For the proper growth of aerobic organisms a half-inch hole should be drilled through the top near the center for a small amount of ventilation to maintain the oxygen supply even during prolonged incubations.

You are now ready to start the regulation of the thermostat for the desired temperature by plugging into the 110 volt current and closing the doors. Keep a frequent watch of the rising temperature and as soon as the desired temperature is attained take notice if the heater bulb is still lighted up, by observing through the ventilator opening in a darkened room or by opening the door slightly. If it is still lighted up open the door and turn the knulled knob of the regulator on the thermostat to the left until the light goes out. Then watch the temperature again. If it should go too low the next time turn the regulator to the right but let sufficient time elapse to insure even distribution of the heat before making any changes in regulation. Let the incubation continue for some time after the right temperature is attained to make sure that an exact adjustment has been made. You have now completed a very efficient and serviceable incubator.

If it is desired to use the incubator on 32 volt current, purchase the thermostat without the heater bulb and replace same with a 100 watt 32 volt bulb and regulate as above.

DETERMINATION OF THE P_H OF NORMAL AND MALIGNANT TISSUES WITH THE GLASS ELECTRODE AND VACUUM TUBE NULL INDICATOR*

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IN A previous communication[1] the authors have discussed the results of P_H measurements with the glass electrode on certain normal and tumor tissues. It is the purpose of this paper to describe the method. The apparatus used requires comparatively little electrical shielding and is simple, stable and semiportable in nature.

METHOD

The method for measurement of the potentials developed by the glass electrode cell was the conventional Poggendorf circuit with the exception that a vacuum tube null instrument was used. A Leeds and Northrup type K potentiometer was used to furnish the known opposing potential.

THE NULL INDICATOR

The present circuit differs from those previously described by the authors[2, 3, 4] mainly in that the bias and free grid-adjusting resistors are in parallel with the power supplies instead of in series. This arrangement gives equal stability with improved sensitivity and simplicity of operation.

Fig. 1 with the list of circuit constants fully explains the electrical values.

OPERATION

Potentiometer *P* is adjusted to furnish correct opposing potentials in the usual manner.

To adjust the null apparatus, batteries *A* and *C* are connected and allowed to operate for twenty minutes or a half hour in order that the various chemical and thermal equilibriums may be established.

With no connection at jack *S*, *Rg* is set so that galvanometer *G* gives no deflection when tapping switch *T* is pressed. Plug *B* is now inserted in *S*, and *Rb* is adjusted until *G* again gives no deflection when *T* is depressed. *B* is removed from *S* and replaced by *E* which completes the external cell-measuring circuit. *P* is adjusted until *G* again gives no movement, and the E.M.F. of cell *HR* is read from *P*.

SHIELDING

Under ordinary conditions when glass electrodes with resistances of less than 15 megohms are used, no shielding of the external circuit is required.

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As indicated in the list of constants the tubes *VV* are shielded electrostatically (and to a slight extent, electromagnetically) to aid in setting *Rg* when the grid circuit is open. The general rule for shielding is obviously that when the electrical strays reach a magnitude comparable with the currents flowing in the input measuring circuit, screening is necessary. A connection to ground is sometimes helpful but for the present work was unnecessary.

SENSITIVITY, STABILITY, LEAKAGE

With the circuit described which includes a portable galvanometer, a sensitivity of from 2,500 to 4,000 mm. per volt is obtained. In other words, when the bridge is out of balance by an amount representing 0.01 P_H a deflection of from 1 to 2 mm. is noted on galvanometer *G*. Tube characteristics and resistance conditions in the input circuit cause considerable variation in this factor.

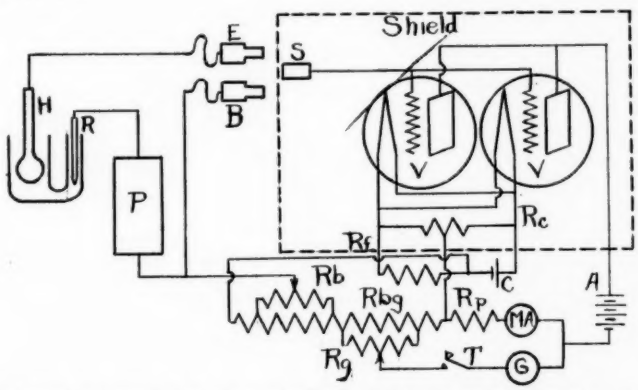

Fig. 1.—Schematic circuit for glass electrode E. M. F. measurements. Circuit constants. *Shield*, Metallic enclosure for tubes to assist in adjusting circuit when the grid is "free." *H*, Haber type glass electrode. Corning Glass Works' 015 glass. Resistance 5-12 megohms. Diameter of bulb approximately 8 mm. *R*, Ball type reference electrode (See text). *P*, Conventional method for securing known potential. *E*, *B*, Plugs of the small radio type. *S*, Jack to fit *E* and *B*. *V*, *V*, "Duovac" 1.1 volt 270 milliampere filament triodes. *A*, Battery of 12 large size flashlight cells, approximately 18 volts. *Rc*, Fifty-ohm midtapped resistance. *Rf*, Filament resistor 1.9 ohms. *C*, Filament supply, 2-volt storage cell of approximately 50 ampere-hour capacity. *Rbg*, Two-hundred-ohm resistor equipped with four movable contacts. *Rb*, *Rg*, Variable resistors of the radio potentiometer type, 500 ohms each. *Rp*, Plate resistor, 3,000 ohms. *MA*, Plate circuit milliampere meter 0-1 or 0-1.5. Not necessary for actual operation but helpful in making adjustments. *G*, Portable pointer type galvanometer, resistance 1,100 ohms; sensitivity 8 mm. per microampere. *T*, Tapping switch.

Mechanically, the apparatus is stable, and for ordinary conditions no cushioned mounting is necessary. Electrically, the drift is very small when the various thermal and chemical equilibriums have been reached—often less than the equivalent of a millivolt per hour. The room temperature, of course, must remain constant since the power supplies have temperature coefficients. That this is a major factor in the drift characteristic was demonstrated by allowing the room temperature to change slowly and taking time-room-temperature and time-drift relationships. The curves obtained were similar in shape with the drift change following the thermometer change with a delay of from twelve to fifteen minutes.

Leakage difficulty is negligible if care is taken to have the connection between *H* and *E* short and well insulated. For the latter purpose, dry wooden

supports have always been found adequate. Results with the equipment when used on a constant system are reproducible to a few hundredths of a millivolt.

ELECTRODES

Haber type electrodes *H* made from Corning 015 glass were used having diameters of approximately 8 mm., and resistances ranging from 5 to 12 megohms. Some of the electrodes were filled with a Clark and Lubs buffer (P_H about 2) saturated with quinhydrone. Others were filled with N/10 HCl (approximately) saturated with quinhydrone.[6]

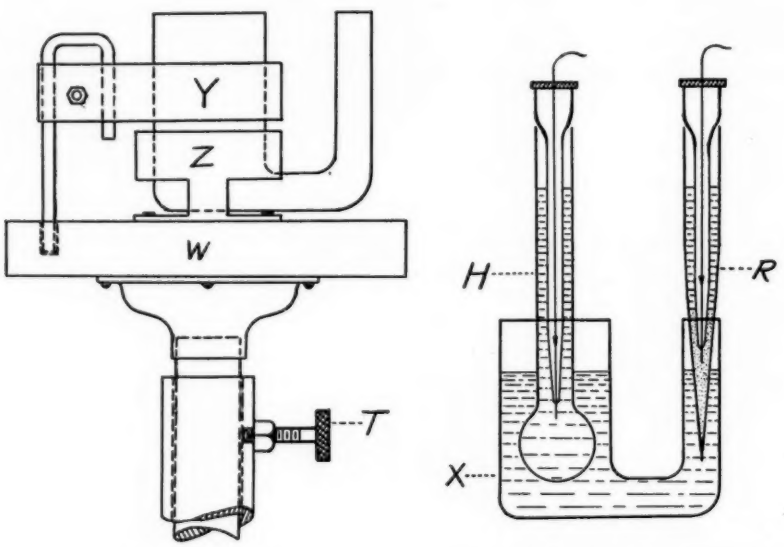

Fig. 2.—Electrode assembly shown in detail. *H* is the glass electrode (see circuit constants for Fig. 1) containing buffer or HCl saturated with quinhydrone; *R* is a calomel Ball type reference electrode. The electrical contact to each electrode is made by a fine platinum wire which is sealed into a glass capillary. A copper wire soldered to each platinum wire makes contact with the measuring device. The electrode vessel *X* is fixed rigidly to the wooden base *W* by the collar *Y* and socket *Z*, both of spring brass. Electrodes *R* and *H* are clamped in position above *X* and contacts with the material to be measured are secured by raising the assembly *WXYZ*. The latter is held in place by thumb screw *T*.

The complete electrode assembly is shown in Fig. 2.

The reference electrode *R* was the general type described by Ball.[8]

The complete electrode combination was as follows:

Pt	Normal Calomel	Buffers or Tissue	Glass	N/10 HCl or Buffer	- Quinhydrone	Pt

CALIBRATION OF GLASS ELECTRODE

The glass electrode was calibrated before and after each determination against Clark and Lubs buffers.[9] The values used for calibration were 6.55, 6.98, 7.14, 7.35, 7.53, and 7.76. These were compared by both the hydrogen and quinhydrone electrodes.

We observed, as did Britton and Robinson,[7] that the glass electrode within this P_H range adheres to its calibration potentials for several hours. These observers found their electrodes in this region to come to equilibrium almost instantaneously, whereas, in our work about four minutes were required before a steady potential was attained. This can hardly be attributed to time to dissipate a charge; for after equilibrium was attained, the glass electrode was removed from solution, then replaced and a reading again taken which was always found to be the same as that at equilibrium.

The electrode was cleaned by washing with distilled water of the same temperature as the electrode. It was dried by carefully wiping with jagged edges of torn filter paper.

P_H OF TISSUES

Different normal and cancer tissues from rats and mice were used. The animal was killed, the tissue immediately removed, quickly macerated and

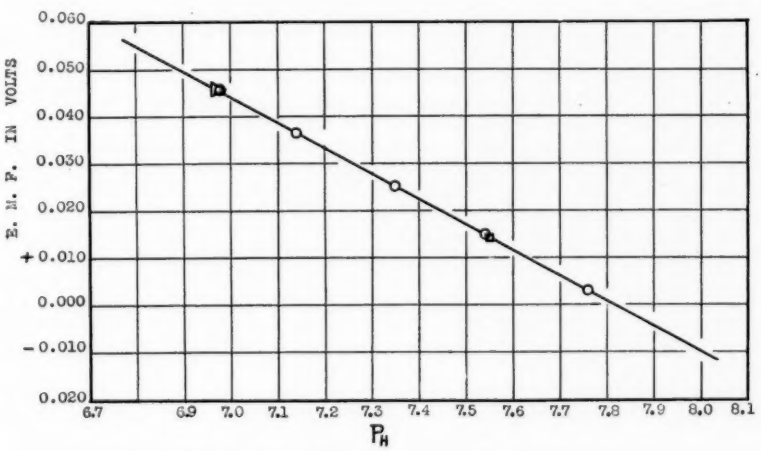

Fig. 3.—Mouse with Tumor 63.

placed in electrode vessel (Fig. 2) and forced into the connecting arm so that contact could be made with the reference electrode *R*. As soon as the glass electrode bulb was completely covered with tissue, a thin layer of paraffin oil was added and E.M.F. measurements taken. The system came to equilibrium within three or four minutes and remained constant for approximately another three minutes, after which there was the usual slow drift toward a lower P_H. The P_H values of the tissues were obtained directly from the calibration curve (see Fig. 3). With the apparatus in readiness the P_H measurements were completed within eight minutes after the death of the animal.

In Tables I, II, and III are some results obtained by the method outlined.

TABLE I

NORMAL RATS

NUMBER	P_H MUSCLE FROM LEG	P_H LIVER
1	6.99	6.78
2	6.89	6.80
3	6.88	6.98
4	6.72	6.88
5	6.70	6.84
Average	6.84	6.86

TABLE II

TUMOR RATS

NUMBER	P_H FLEXNER-JOBLING RAT CARCINOMA	P_H NORMAL MUSCLE FROM LEG	P_H LIVER
1	7.34	6.83	7.05
2	7.39	6.87	6.93
3	7.41	6.92	6.95
4	7.29	–	6.87
Average	7.36	6.87	6.95

TABLE III

MICE WITH TUMOR 63

NUMBER	TUMOR TISSUE	NORMAL MUSCLE FROM LEG
1	7.54	6.97
2	7.39	6.78
3	7.24	6.81
Average	7.39	6.85

Furusawa and Kerridge,[10] and Carlström, Ege, and Henriques[11] obtained average P_H values of 6.89 and 6.88, respectively, for tissues from leg muscles of normal rats. Our results for similar normal tissues are therefore in close agreement.

SUMMARY

A method has been described for the determination of P_H in normal and malignant tissues by means of the glass electrode. The electrical apparatus is comparatively simple, stable and semiportable. For the normal animal tissues compared, our results are in close agreement with other workers.

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THE RECORDING ELECTRODYNAMIC BRAKE BICYCLE ERGOMETER*

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WE HAVE elsewhere briefly described a bicycle ergometer which has since been rebuilt, further improved, and modified.[1] It fulfills the need for a work machine which not only provides a satisfactory brake and accurately measures work done, but which simultaneously records the constancy of the output and the speed of working. These are factors of importance in physiologic studies of exercise so far as the cost of muscular work is related to the speed and steadiness with which it is performed. The constancy of the output is a variable hitherto unmeasured by the ergometers in common use. The chief purpose of this communication is to present the circuit diagram of the new recording electrodynamic brake ergometer and to describe its method of calibration.

Principle.—The principle of the electrodynamic brake resides in the fact that a separately excited direct current generator is the load. When a generator armature is rotated and the field is excited, a voltage is generated. If the armature is then connected to a resistor, a current will flow. This current reacting against the field magnetism develops a counter torque or braking effect. The work done in unit time is the torque multiplied by the distance it moves, or the rate of work in watts is the generated voltage times the current flowing.

Description of the Apparatus.—As constructed, the ergometer consists of a standard woman's bicycle frame from which the wheels and handlebar have been removed, mounted on a portable supporting structure along with the necessary accessories. These consist of a generator, storage battery, load resistor, field control resistor, ammeter, voltmeter and electrical revolution counter. The set-up is illustrated in Figs. 1 and 2.

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The generator is a 12 volt Dodge automobile starter. The series winding is disconnected and the shunt winding is connected for separate excitation. The generator is directly driven from the main sprocket which has 100 teeth. There are 10 teeth on the generator sprocket, giving a generator to pedal speed ratio of 10. A fly wheel is affixed to the generator shaft to assist in the maintenance of constant speed.

The load resistor is of advance wire which has a resistance practically independent of temperature. The resistor is connected across the armature

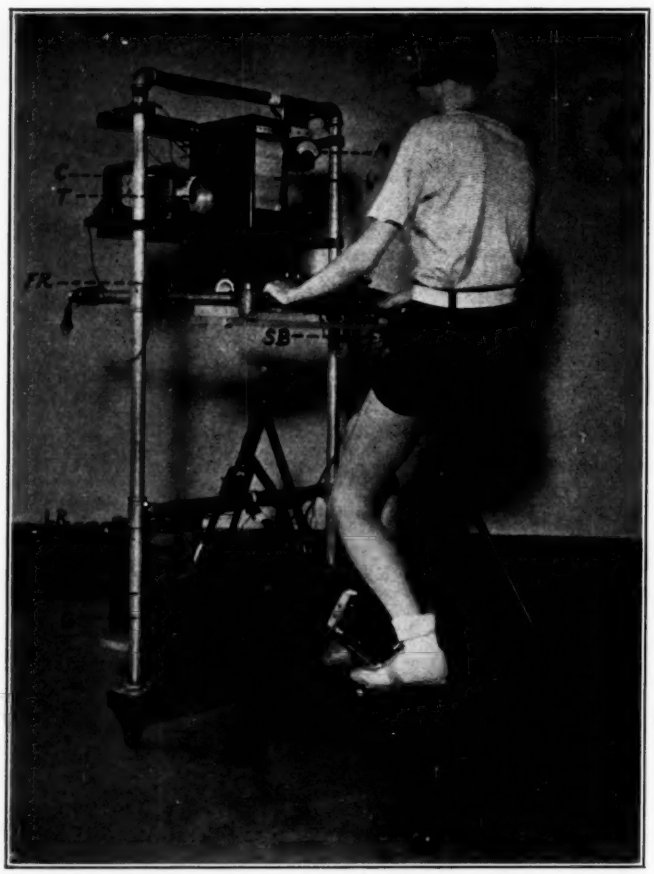

Fig. 1.—Photograph of the recording electrodynamic brake bicycle ergometer. *A*, ammeter; *V*, recording voltmeter; *T*, timer; *C*, electric clock; *FR*, field control resistor; *SB*, switch board; *LR*, load resistor; *F*, flywheel; *G*, generator; *MS*, main sprocket. The rotating switch for the electrical revolution counter may be seen just above the hub of the main sprocket. The storage battery and charger are located to the right of the generator and the flywheel.

with a switch S_3 which may be opened for testing purposes. It is made up of 6 coils of wire in parallel so that the value of the load may be changed for subjects of widely different strengths.

The voltmeter is a 15 volt graphic recording instrument. The clock is driven by a Warren self-starting synchronous motor, and has chart speeds of 3 inches per minute and 3 inches per hour. It is started and stopped by switch S_4 which is mounted within easy reach of the subject. The lever that

changes the chart speed may also be reached by the subject. The recording needle is large and easily visible, so that the meter is distant reading at all times and may be made recording at will.

A 12 volt storage battery is used to supply the current for the field excitation, the revolution counter and the timer. For convenience a portable charger is mounted on the platform which holds the battery, and it may be charged during long riding bouts. The circuits are so arranged that the battery may also be used to furnish power to operate the generator as a motor for calibration purposes.

The ammeter is a 15 ampere distant reading instrument. It indicates the value of the excitation current when the ergometer is in use and the value of

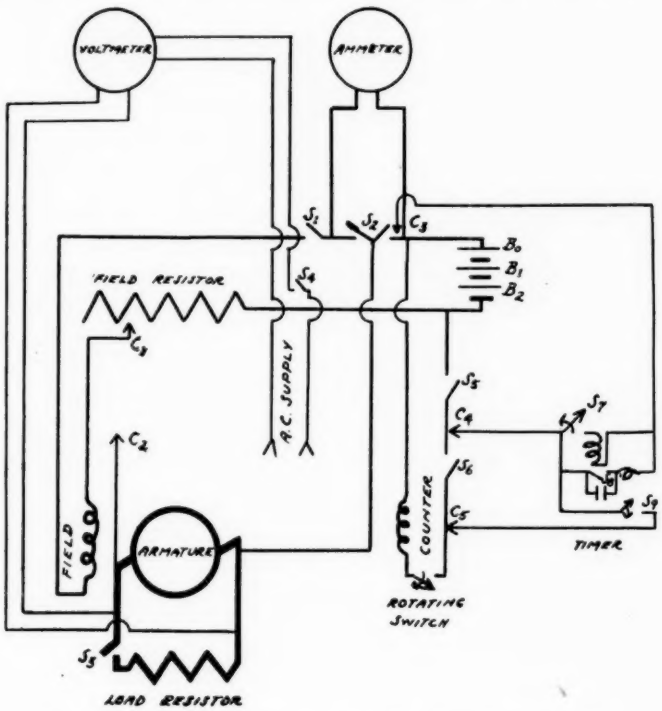

Fig. 2.—Circuit diagram of the electrodynamic ergometer.

the current to the armature for motor action during calibration procedures. Field currents from 1 to 7 amperes are those normally used. Switch S_1 is in the field circuit. The double throw switch S_2 is used to send the armature motor current direct to the armature, or through the ammeter in parallel with the field current. The ammeter may thus be made to indicate either the field current alone, or the combined field and motor armature currents.

The field current is controlled by a 20 inch, 12 ampere, 8.5 ohm resistor with a sliding contact, C_1. This field resistor is mounted in front of and close to the operator, so that it may be reached and controlled while riding. The roving contact, C_2, may be connected to various points on the battery for motor operation purposes.

The revolution counter is operated by an electromagnet. A rotating switch on the main sprocket makes contact once each revolution. With switch S_6 closed, S_5 starts and stops the counter. It may be automatically kept in and out of service by a timing device parallel with S_6. A three conductor cable connects the timer with the ergometer. The contacts are made as indicated by C_3, C_4, and C_5. When S_6 is open, the timer is in control of the counter, but when S_6 is closed, the timer is inoperative. With S_5 open, both the counter and the timer are out of service.

In the timer itself, S_7 is a switch operated by an electric clock and closed every fifteen seconds. The current through S_7 operates a relay that closes S_8. The current through S_8 operates an electromagnet that rotates a drum $\frac{1}{100}$

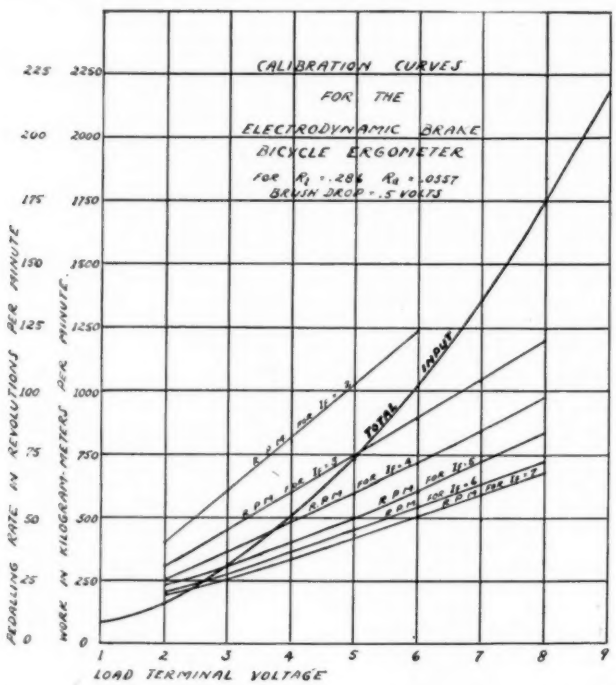

Fig. 3.—Curves for the conversion of load terminal voltage into rate of working and for the estimation of pedalling rate under various field current and load terminal voltage conditions.

of a revolution. The switch S_8 is paralleled by a condenser to cut down sparking. The contact on the drum, S_9, is opened and closed for different lengths of time depending upon the calibration of insulating strips on its surface.

Calibration of the Ergometer.—The output of the subject is the input to the ergometer. This input is composed of the stray power losses and the armature generated power. For any speed and field current and no armature current, a certain amount of power is required to run the ergometer. This power is assumed to be independent of armature current and is called the stray power loss. The armature developed power is the product of the armature current and the generated voltage. It is possible to determine the value of these losses for any load terminal voltage. Their sum plotted against load

terminal voltage is the calibration curve of the instrument. This is presented in Fig. 3, the speed vs. load terminal voltage curves being plotted on the same sheet.

The stray power losses are determined by making a series of runs, varying the speed but keeping the field current constant for each. The speed, field current, armature voltage, and armature current are recorded. The stray power losses are then calculated and the corresponding load terminal voltage determined. The stray power losses for the various speeds and field currents are then spotted on a graph and a composite curve drawn which gives the loss for any voltage.

The armature developed power is calculated in the following way: The generated voltage is the sum of the load terminal voltage, the brush drop, and the resistance drop of the armature proper and the leads. The armature current is the load terminal voltage divided by the load resistance. The load resistance is constant and is independent of temperature. The brush drop is assumed constant at 0.5 volt. The resistances of the armature and leads vary with the temperature, but since they are small, the error due to temperature change is small.

The resistance of the armature, the leads and the load resistor was determined, employing the volt ampere method, and using currents over the range that would be obtained under load conditions. The brush drop was estimated by plotting a series of curves of no load terminal voltage vs. speed, for constant field current. These curves were projected back to the zero speed line, their intersections with this line being taken as the brush drop. In making the no load runs the generator was operated as a motor. Switch S_3 was opened, S_2 was closed up, S_1 was closed, the field current I_f was adjusted to some value, and contact C_2 was connected to some point on the battery, as B_1. The apparatus now operates motor action at no load, and the input to the armature is the stray power losses plus a small loss due to brush drop and armature resistance. The field current was again adjusted and the values B, E_t, I_f, and R.P.M. were recorded. S_2 was closed down. The armature and field currents both pass through the ammeter. They were recorded as $I_{(a+f)}$. S_2 was closed up and a new contact made on the battery, and the data were obtained over the range of speeds to be used. Then the entire procedure was repeated for all values of field current over the range to be used.

The stray power loss is the generated voltage times the armature current. The generated voltage is related to the no load terminal voltage by the following equation: $E_g = E_t - (0.5 + I_a R_a)$.

The no load data for the stray power calculations were arranged as follows:

$$B \quad E_t \quad I_f \quad I_{(a+f)} \quad I_a \quad \text{R.P.M.} \quad \text{S.P.} \quad E_{load}$$

where B is the connection on the battery, E_t is the no load terminal voltage, I_f is the field current, $I_{(a+f)}$ is the sum of the field and armature currents, I_a is the no load armature current, R.P.M. is revolutions per minute, S.P. is stray power loss, and E_{load} is the load terminal voltage for a generated voltage of the same value as the generated voltage for the corresponding E_t.

For any stray power loss the generated voltage is calculated as before. The load terminal voltage is related to the generated voltage by the following equation:

$$E_g - 0.5 = E_t \left(1 + \frac{R_a}{R_{load}}\right)$$

The armature developed power is the product of the armature load current and load generated voltage. This is expressed in terms of load terminal voltage by the following equation:

$$W = \frac{E_t^2}{R_{load}} (R_L + R_a) + \frac{0.5\ E_t}{R_{load}}$$

The watts are changed to Kg.m./min. by the following equation:

$$\text{Kg.m./min.} = W(6.15075).$$

Criticism of the Instrument.—The possible sources of error are, (a) stray power losses, (b) armature I^2R losses, and (c) brush drop loss. These are all small and their possible variation from the mean is also small. Their total effect is assumed to be less than errors due to the inability of the subject to keep the voltage constant, and may therefore be neglected.

The validity of this instrument has not been determined. It purports to measure the output of mechanical work performed by human subjects. The comparison of the results obtained with some outside criterion is difficult to effect, and further, is unnecessary because the instrument is built upon a brake principle of proved characteristics already demonstrated and long used with success in engineering.

The degree to which the measuring devices for the determination of mechanical work, constancy, and speed give invariable readings under identical conditions is high. For a given field current and pedalling rate, the load terminal voltage is always the same, irrespective of the environmental conditions and the experience of the operator. Constancy and speed are measured by synchronized, electrically driven clocks. Under identical field currents and load terminal voltages, the speed of pedalling may be well duplicated, both during protracted bouts of exercise and during pieces of work performed on different days. For example, at a given field current an experienced rider holds the voltmeter needle at the prescribed point with such accuracy that the speed of pedalling, estimated for one minute at five-minute intervals during a one-hour period of exercise, will not vary by more than one revolution per minute plus or minus, and will remain at the modal R.P.M. 83 per cent of the time.

We believe this measuring device is especially good because of its objectivity and administrative economy. The recording voltmeter graphs the constancy of the performance and automatically records the data necessary for the calculation of the rate of working. The speed of pedalling is also automatically counted at any desired intervals during the exercise. The apparatus is movable and may be used by a subject without the assistance of an operator.

Principles for the Selection of Load and Speed Conditions.—Table I presents the rate of working and the speed of pedalling under a wide range of

field current and load terminal voltage variations. The exercises performed under these loads and speeds have been classified physiologically as to their severity. In Table II this classification is presented along with the data for performing the different exercises in various gradations on the electrodynamic brake bicycle ergometer.

TABLE I

THE RATE OF WORKING AND SPEED OF PEDALLING UNDER VARIOUS CONDITIONS OF FIELD CURRENT AND LOAD TERMINAL VOLTAGE*

LOAD TERMINAL VOLTAGE	RATE OF WORKING	SPEED OF PEDALLING AT VARIOUS FIELD CURRENTS					
		2 AMP.	3 AMP.	4 AMP.	5 AMP.	6 AMP.	7 AMP.
	Kg.m./Min.	R.P.M.	R.P.M.	R.P.M.	R.P.M.	R.P.M.	R.P.M.
2	167	40	32	26	23	20	19
3	309	60	45	36	32	28	25
4	501	78	59	48	41	38	34
5	740	104	73	58	50	47	43
6	1,031	124	88	72	60	55	51
7	1,366		104	84	74	64	60
8	1,749		120	98	84	72	68

*For a load resistance of 0.286.

TABLE II

SPEED AND LOAD CONDITIONS FOR GRADATIONS OF THE VARIOUS TYPES OF EXERCISE

RATE OF WORKING	SPEED OF PEDALLING	FIELD CURRENT	LOAD TERMINAL VOLTAGE
		Mild Exercise	
Kg.m./min.	R.P.M.	Amperes	Volts
167	40	2	2
167	32	3	2
309	45	3	3
309	36	4	3
		Moderate Exercise	
309	60	2	3
309	45	3	3
501	78	2	4
501	59	3	4
740	73	3	5
501	48	4	4
		Violent Exercise	
740	104	2	5
1031	88	3	6
1031	124	2	6
1366	104	3	7
1366	84	4	7
1749	120	3	8
1749	84	5	8
1749	98	4	8
		Exhaustive Exercise	
740	73	3	5
1031	88	3	6
1031	72	4	6
1366	84	4	7
1366	74	5	7
1749	84	5	8
1749	72	6	8

The exact load and speed conditions to be selected depend upon the age and the development of the individual subject. The child does not possess the muscular strength to perform exercises at the upper ranges of field cur-

rent. Nor can the upper ranges of speed be well borne. The young adult is better suited than the subject in any other age group to carry on violent exercises of speed. He can be expected to perform creditably under the low field current and high voltage conditions associated with the highest pedalling rates. Muscular power is at its height during middle age and the exhaustive exercises of endurance at high voltage and field currents are then well tolerated. Table II is based upon the performance of strong, healthy, young adult women in training for physical education. At a given rate of working, they invariably prefer an exercise with a low field current and high speed of pedalling to one with a high field current and a slower pedalling rate. Exercises of speed are performed well, but these subjects do not possess the muscular power to pedal against a strong resistance. The limitations of the table are obvious. Mild exercise for one individual may be moderate for another. Under certain speed and load conditions an exercise may be easily borne for ten minutes, but carried on for an hour, it becomes exhaustive.

Operation of the Ergometer.—Having decided upon the field current and the load terminal voltage with the aid of the tables, close switch S_3 and switch S_1, leaving C_2 disconnected. Adjust the field current to the proper value by moving the slide contact on the field resistor. The proper selector on the timing device is connected at S_9 and the drum is rotated until it has at least one movement to make before S_9 contacts. The roll mechanism of the voltmeter is started by closing the clock motor control switch S_4. The revolution counter is read, pedalling the bicycle is begun, and S_5 is closed. The subject now has fifteen seconds or more to get to stable conditions before S_9 contacts and the revolution counter begins to operate. The counter will operate for the chosen time and will then be automatically disconnected. The reading of the counter is then recorded, and the subject continues to ride and record to the end of the run.

Comment on the Uses of the Instrument.—The ergometer has thus far been used for the following four types of problems: (1) The repetition of identical work under constant physiologic conditions for the study of training. (2) The repetition of identical work under variable physiologic conditions for the study of their effect on its cost. (3) The study of the physiologic effect of varying speed at a constant mechanical output. (4) The graphic record of fatigue curves. The advantages of the recording ergometer for repetitious work are obvious. The instrument lends itself especially well to studies necessitating variations in the speed of pedalling at constant output. A single manipulation, the alteration of the field current, accomplishes this since the rate of working remains constant as long as the load terminal voltage is unchanged.

SUMMARY

The electrodynamic brake principle has been applied to a stationary bicycle ergometer. It has furnished an easily adjustable brake with a constant coefficient of friction. The introduction of a recording voltmeter has made it possible to measure with accuracy the rate and steadiness of working. The

instrument may be used under a variety of speed and load conditions without the assistance of an operator.

The ergometer was built by J. S. Hipple, mechanician, in the shops of the Medical School. We wish to acknowledge his helpful suggestions and cooperative assistance in the development of this project.

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THE TRYPTOPHANE CONTENT OF BLOOD SERUM*

A New Technic

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MITCHEL and Hamilton[1] in their work on the amino acids quote J. M. Looney, as follows: "The multiplicity of the methods proposed for the estimation of tryptophane and the inconsistency of the values given are sufficient evidence of the importance attached to the subject and the worthlessness of most of the methods." Such a situation offers little encouragement to present another tryptophane method, but it does suggest that conflicting results in differentiating between normal and pathologic material might be avoided by some standardization of procedure. The tryosine content of the plasma proteins has been shown to be constant, and the determination of this amino acid can, therefore, be used as a measure of the total protein. Tryptophane, however, in the light of our present knowledge occupies a position more characteristic of an independent variable. Glycocol, alanine, serine, cysteine, aspartic acid, and glutamic acid are among the glucose formers of the amino acids. Valeine, leucine, isoleucine, phenylalanine and tyrosine give rise to beta-hydroxybutyric acid. Histidine and tryptophane are exceptions. It has been shown that tryptophane forms neither glucose nor beta-hydroxybutric acid in the organism of the dog. The tryptophane content of the plasma proteins has not been accurately established, and the practical application of a blood serum tryptophane method does not, therefore, at the moment seem apparent. The lack of such a present application, however, should not deter us from keeping our faculties of observation alive and seeking to learn what we can of physiologic or pathologic significance about this unique amino acid. It is with the hope that it may be used for such a purpose that the following technic for blood serum tryptophane is suggested.

*From the U. S. Veterans' Administration Hospital, Palo Alto, California.
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The specificity of the Adamciewicz reaction for tryptophane is entirely beyond question, and as applied in the Hopkins and Cole Glyoxylic test it has been shown to be quantitative within wide limits. It is particularly suitable for clinical laboratory application, as it is simple and rapid. I have developed its application to human blood serum and prepared an artificial color standard which is practically permanent if handled with ordinary care. The use of such a standard is open to criticism from the standpoint of absolute values, but aside from its comparatively negligible expense it has a distinct advantage over the chemical standard, which is notably unstable as a means for the accumulation of data. The chemical standard being subject to deterioration an element of error would likely creep into any series for which it might be employed, whereas with the artificial standard, assurance is had that the basis of comparison will not change throughout the series.

STANDARD

The standard is prepared from stock 0.05 per cent aqueous solutions preserved with a crystal of thymol of the DuPont water soluble dyes violamine blue and pontamine fast blue. Exactly 2.8 c.c. of each of these two stock solutions is diluted to 100 c.c. with a solution consisting of 3 per cent gelatin, 0.9 per cent sodium chloride, 5 per cent oxalic acid, and several crystals of thymol dissolved by heating on a boiling water-bath, cooling, and clearing by sedimentation, centrifugation, or filtration. A good grade of gelatin must be used and the diluting solution come through crystal clear with a trace of amber color. The DuPont dyes, which were developed specifically for use as color standards, are of absolutely uniform color value. They are light fast over a period of years if the solutions are kept in dark bottles. The gelatin vehicle is used to provide in the standard a molecular density, approximating that of the unknown blood serum mixtures. This standard may be prepared in the laboratory or may be purchased ready for use from the Hynson, Westcott and Dunning Company of Baltimore, Md., to which firm I am indebted for acquainting me with the properties and use of the DuPont water soluble dyes.

REAGENTS

Hopkins-Cole Glyoxylic Reagent.—To one liter of a saturated solution of oxalic acid add 60 gm. of 2 per cent sodium amalgam and allow the mixture to stand until the evolution of gas ceases. Filter and dilute with 2 or 3 volumes of water.

The glyoxylic reagent prepared according to Benedict's formula gives practically identical values with the above, but I have found it not to keep as well and old reagents to give less brilliance of color.

Sulphuric Acid 95 per cent.—A chemically pure or reagent quality of concentrated acid must be used, the usual U. S. P. grades giving cloudy solutions.

TECHNIC

a. Clear blood serum is diluted 1/10 with distilled water and 1 c.c., equivalent to 0.1 c.c. of serum, placed in a clean glass test tube.

b. Add 1 c.c. of glyoxylic reagent and then 3 c.c. of sulphuric acid, a few drops at a time, while shaking the tube.

c. Let stand for several minutes for the maximum color to develop and the mixture to cool somewhat and then compare in the colorimeter with the standard set at 15 or 20.

Calculation: $\frac{\text{Reading of Standard}}{\text{Reading of Unknown}} \times 100 =$ mg. of total tryptophane per cent of blood serum

The standard will be found to be slightly more brilliant photometrically than most of the blood specimens but to provide a satisfactory basis for comparisons. The unknown solutions being strongly acid and quite hot must be handled carefully.

TABLE I

RECOVERY OF ADDED TRYPTOPHANE

SPEC. NO.	ORIGINAL SPEC. IN MG. PER CENT	TRYPTOPHANE ADDED IN MG. PER CENT	TRYPTOPHANE RECOVERED IN MG. PER CENT	PER CENT RECOVERED
1	167.0	100.0	250.0	83
2	181.8	100.0	285.7	104
3	148.1	100.0	259.7	112
4	190.4	100.0	285.7	95
5	166.7	50.0	212.9	92
6	200.0	50.0	250.0	100
7	153.8	50.0	200.0	92
8	166.7	50.0	210.5	98
9	178.5	25.0	200.0	86
10	190.0	25.0	210.5	80
11	181.8	25.0	206.1	97
12	153.8	25.0	181.8	112
		Average per cent recovery		96

The appended table reports some recoveries of pure tryptophane added to sera in the diluting water (Table I).

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A COMBINATION CONDENSING AND RECEIVING VESSEL*

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DURING the past few months the authors have had occasion to make a number of protein analyses. Since only small amounts of material were available, Cavett's[1] micromodification of the Van Slyke method was used. In the determination of arginine, an aliquot of the basic fraction is hydrolyzed with strong NaOH, which converts arginine into ornithin and urea. After hydrolysis, ammonia is distilled into standard acid. In the original method of Holm[2] the ammonia is distilled through a condenser, but in Cavett's modification, it is distilled into a receiving tube containing standard acid. A large test tube is usually employed as a receiver and must always be placed in a beaker of ice in order for it to function as a condenser. Although this method gives satisfactory results, it is not convenient, and necessitates a supply of ice. Consequently, a combined condensing and receiving vessel was constructed which functions better and which uses circulating water as the cooling agent. The construction of the vessel is shown in Fig. 1.

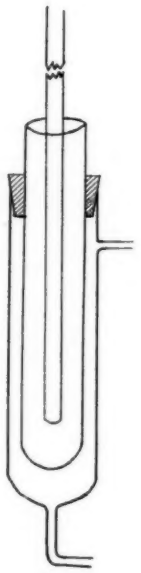

Fig. 1.

The condenser shell was constructed from glass tubing 5 cm. in diameter and 22 cm. in length. A water inlet was placed at the bottom of the tube and

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an outlet was placed 4 cm. from the top of the tube. The receiving vessel was a pyrex test tube 3 cm. in diameter and 20 cm. in length. A rubber stopper (size No. 9) was bored to receive the test tube.

The standard acid is placed in the tube and the distillation conducted in the usual way. A current of tap water cools the test tube, which serves as a condenser. When the distillation is complete, the test tube may be removed and the solution titrated.

The authors have secured satisfactory results with this apparatus. It has been used for the determination of arginine in protein hydrolysates, and in the microdetermination of ammonia in biologic fluids and in bacterial cultures. It could also be used in micro-Kjeldahl determinations, eliminating a special condenser.

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DOG-OPERATING TABLE*

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SEVERAL months ago I was confronted with the task of outfitting a laboratory for dog surgery. A definite problem was encountered in connection with securing suitable dog-operating tables. After some preliminary experimentation with the various types of tables with which I was familiar, all of which proved to present disadvantages, it was decided to attempt to design a more satisfactory type of apparatus. The design of the dog-operating table which was finally developed and which experience at the Louisiana State University Medical Center has shown to be particularly useful and convenient, is herewith submitted in the belief that it embodies features which may be of interest to others working in the educational and laboratory fields.

The characteristics which were stressed in designing the table were: (1) So far as possible, an all metal construction; (2) portability, such that the table could be readily handled by one person; (3) quick and easy disassembly of the entire apparatus; (4) absence of all pockets and depressions in which dirt might accumulate; (5) accessibility of all parts for easy cleaning with soap and water; (6) a wide range of utility; and (7) sturdiness and stability.

The table is constructed of pipe iron, angle iron, and monel metal, the only wood used in its construction being two monel metal covered boards which constitute a troughlike support for the animal's body.

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Dimensions: Fig. 1 shows the completely assembled table. The over-all dimensions of this table, exclusive of the instrument tray (which is removable) are as follows: height 40 inches, length 47 inches, width 16½ inches. Fig. 2 is a photograph of the bare frame of the table. For the most part this frame consists of pipe iron, of 1 inch diameter, outside measure, the pieces being cut to length and solidly welded together. The over-all dimensions of the frame of the table (exclusive of the instrument tray) are: height 36½ inches, length

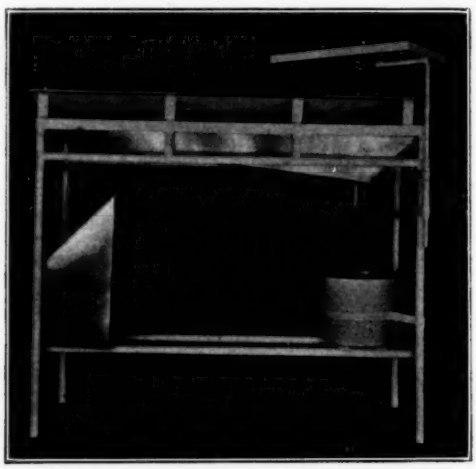

Fig. 1.

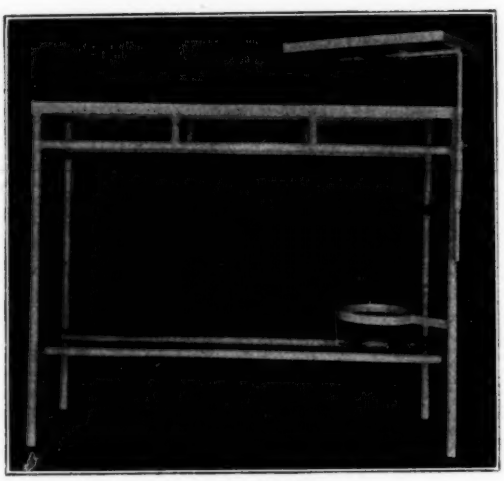

Fig. 2.

46 inches, width 16 inches. The extreme top of the frame is made of angle iron which measures 1¼ inches on a side. The angle iron portion of the frame is open at the extreme right end, for the insertion of a monel metal pan, subsequently to be described, which slides in and out on the lower surface of the angle iron, like a drawer. The cross braces of pipe iron at the bottom of the table are ten inches on center from the floor. The end cross braces of pipe iron at the top of the table are respectively 25½ inches (right end of the table),

and 31½ inches (left end of the table), on center from the floor. The side braces of pipe iron at the top of the table are 32½ inches on center from the floor. At the right end of the table, above the cross braces nearest the floor, there is an arrangement made of strap steel, for the reception of a white enamel ware bucket. This consists of a ring suitably braced both to the legs of the table, and also to the base on which the enamel ware bucket rests. The base for the bucket is provided by two cross pieces of strap steel, welded to the lower cross braces of the table. The steel used in this part of the construction is 1 inch wide, and ⅛ inch thick. All joints are firmly welded.

The instrument tray measures 17½ by 13 inches. The frame of the tray consists of angle iron, welded at the corners in the form of a rectangle. The angle iron measures one inch on a side. Two cross braces of strap steel are welded across the bottom of the frame of the tray, and flush with its lower surface. They measure 1 inch in width and 3/16 inch in thickness. The tray

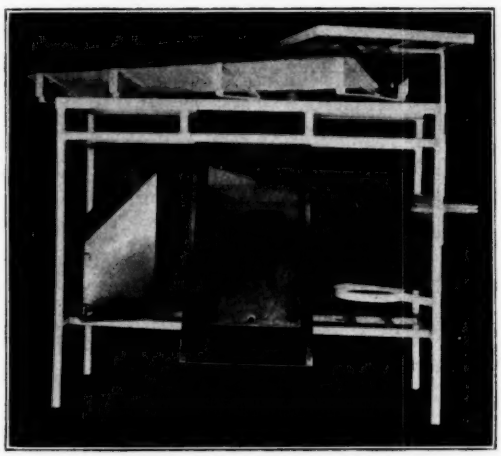

Fig. 3.

is supported by a right-angled steel rod. The horizontal limb of this rod measures 7 inches in length, and is welded both to the midportion of one end of the angle iron frame of the tray, and also to the midportion of the proximal strap-steel crossbar. The vertical limb of the rod measures 22 inches in length and 9/16 of an inch in diameter. The vertical limb of the rod provides support for the instrument tray in the following manner: A tubular piece of pipe iron, 6 inches in length is welded to one leg of the table by the intermediation of two offset pieces of strap steel, 1 inch in length and 3/16 of an inch in thickness. Through this piece of pipe iron, the vertical rod of the instrument tray passes in a snug fit. The tray is made adjustable for height by means of a collar and set screw, the position of which can be varied at will. The distance between the floor and the lowermost extremity of the offset pipe iron support for the instrument tray, is 26 inches. The instrument tray is not only adjustable for height, but swings freely on its supporting rod through an angle of 360 degrees.

Fig. 3 shows the frame of the table together with the accessory parts. These consist of (1) a monel metal instrument tray which fits inside of the instrument tray frame previously described, (2) a sheet monel metal drip pan, which slides in the groove provided by the angle iron top of the table, (3) a monel metal cabinet for the reception of ether cans, masks and other incidental paraphernalia connected with the anesthetization of the animal, and (4) a monel metal, wood, and strap-iron top constructed in a V-shape on which the animal lies.

The instrument tray pan is made to fit snugly into the angle iron tray frame, previously described. It measures slightly less than 17 inches in length, and slightly less than 13 inches in width, and is one inch deep. The edges are neatly turned over a heavy wire frame. The tray is neatly soldered so that it does not leak.

The drip pan is constructed of monel metal, and is of such dimensions that it provides a good sliding fit for the angle iron top of the table. Two flanges, ⅞ of an inch in width are provided on the edges at the top of the pan, one projecting from either side, on which the pan slides. The pan is 1½ inches deep at its shallower end, and 6 inches deep at its deepest part. The bottom of the pan slopes considerably at both ends, and also slightly on both sides. At the center of the extreme lower edge of the pan is soldered a drain spout which is 2½ inches in length, and ¾ of an inch in diameter. This extreme lower edge of the pan is 6½ inches from the end of the table, and the drain spout centers accurately over the receptacle for the enamel ware bucket—or over the bucket itself when the bucket is in place. The upper edge of this drip pan is turned neatly over a heavy wire frame. The pan is neatly soldered so that it does not leak, and inasmuch as its sides slope in all directions, it drains very efficiently.

The cabinet for anesthetic paraphernalia is made of monel metal. It is 12½ inches wide, and 9 inches from front to back. The back of the cabinet is 18 inches in height. The sides of the cabinet at the top slope toward the front which is 9½ inches in height. The edges of the top of the cabinet are neatly turned over a heavy wire frame. The front of the cabinet projects for a distance of 1¼ inches in the form of a flange at the sides, the dimensions of the front of the cabinet being 14½ inches in length, and 9½ inches in height. The flanges fit against the legs of the table, and prevent the cabinet from being displaced backward or inward. Riveted to the back of these projecting flanges, are a pair of strap metal hooks, one placed above the other, and one pair on either end. These hooks engage studs which project inward from the legs of the table, and prevent the cabinet from being accidentally displaced forward or outward. The cabinet is easily removable, however, by raising it straight up, thus disengaging the hooks from the studs. The cabinet rests on the cross supports of pipe iron at the bottom of the table. It is neatly soldered, so that it will not leak.

The top of the table on which the animal rests consists of a V-shaped trough, fashioned from two monel metal covered wooden boards. These boards measure 8 inches wide, 47½ inches long, and ⅞ of an inch in thickness.

As previously mentioned, the two boards form a trough, being separated throughout by a distance of 1½ inches. Four similar supports of angle iron bolted at regular intervals to the bottoms of the monel covered boards maintain the angle of the trough, and support this trough on the extreme edges of the top of the table frame. These angle iron supports are made of 1 by 3/16 inch cold rolled steel stock, bent on a template. They consist of a horizontal bar to which is welded at three places an inverted W-shaped piece of steel. The table top rests on the extreme upper edge of the angle iron frame of the table proper. The nature of the construction prevents displacement of the top of the table from side to side. Metal stops welded to the extreme end of the frame of the table proper, at its open end, prevent the top of the table from becoming displaced in that direction. The top of the table is, however, readily lifted, vertically.

All iron and steel parts of the table are finished with several coats of white enamel.

In operation, the table is extremely satisfactory. Not only is it pleasing to the eye, but all parts are easily removed without necessarily disturbing any other part of the table. The anesthetist's cabinet is disengaged by simply lifting it, and it can be emptied and cleaned with soap and water. The drip pan slides freely from the end of the table without disturbing the trough on which the dog lies. This pan is made entirely of rustless metal, and can be scrubbed into a condition of perfect cleanliness with soap and water. The instrument tray and instrument tray frame can be disassembled by a simple lifting movement. Similarly, the white enamel bucket, although it is entirely stable in its ring stand, can be readily lifted from its position and thoroughly cleaned. The top of the table, consisting of the trough in which the animal lies, although it is thoroughly stable, with respect to end and sidewise motion, can be readily lifted vertically, together with the animal if desired, without disturbing the rest of the ensemble.

NOTE.—This table was skillfully constructed jointly by the pipe-fitting, sheet metal working, carpenter, and paint shops of the Charity Hospital of New Orleans to my plans and specifications, and I am indebted to the mechanics for certain constructional modifications of the original design. The cost of the completely finished tables was $39.60 apiece.

ESTIMATION OF PLASMA BILIRUBIN*

A Comparative Study of the van den Bergh and the Thannhauser and Andersen Procedures

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THE purpose of this communication is to record a series of quantitative determinations of plasma bilirubin by the van den Bergh and the Thannhauser and Andersen technics, and to compare the two methods *with respect to their applicability in clinical work.*

Van den Bergh[1, 2, 3] noted that with his procedure the protein precipitate, formed by the addition of alcohol to serum, may, at times, carry with it considerable amounts of bilirubin and, as a consequence, values obtained for serum bilirubin may be low. Such loss of bilirubin, however, did not occur or was negligible when sera contained no "direct" bilirubin. Thannhauser and Andersen[4] attempted to eliminate this source of error by coupling the bilirubin with the diazo reagent before precipitation of proteins with alcohol. Thus, according to the van den Bergh technic,[5] the serum (or plasma) proteins are first precipitated with alcohol and the protein-free portion is treated with the diazo reagent; whereas, with the Thannhauser and Andersen procedure[4] the diazo reagent is added to the serum (or plasma) *before* precipitation of the proteins. Thannhauser and Andersen also suggested the addition of ammonium sulphate to ensure complete precipitation of protein. Comparison of the values obtained by these two methods[4, 6, 7, 8, 9] indicates that loss of bilirubin by occlusion in the protein precipitate is considerably reduced by the Thannhauser and Andersen technic.

In this Clinic, the van den Bergh method has been used since 1922. Over five thousand determinations have been made, and correlation between plasma bilirubin values and clinical findings has been satisfactory, with one exception; in cases of subsiding jaundice, there appears to be no relationship between the color of the skin and the amount of bilirubin in the blood. This absence of relationship was thought to be due to the rate at which bilirubin is liberated from the tissues in this condition; at times, liberation of bilirubin from the tissues may be slow and the rate at which the pigment enters the blood may be much lower than the rate at which it can be removed. This would explain the frequent finding, in such cases, of jaundice of the skin in spite of normal or nearly normal amounts of bilirubin in the blood. In view of the definitely established fact that the van den Bergh procedure may not be quantitative when the blood contains "direct" bilirubin, it is necessary to seek an explanation of the generally satisfactory results with this technic in

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this Clinic. I was, therefore, prompted to compare the van den Bergh procedure with that of Thannhauser and Andersen and correlate the data with clinical findings.

METHODS

Oxalated plasma was used and all tests were commenced within one hour of collection of blood. Van den Bergh's cobalt-sulphate standard was used.[6] All determinations were carried out by the writer.

Van den Bergh Technic.—Two volumes of 95 per cent alcohol were well mixed with one volume of plasma and the mixture was centrifuged. To 1 c.c. of the clear supernatant fluid was added 0.5 c.c. of 95 per cent alcohol and 0.25 c.c. of freshly prepared diazo reagent. Fifteen minutes were allowed for full color development. The solution was then diluted with 66 per cent alcohol until the color matched that of the standard. In estimating the bilirubin, a dilution factor of 1 in 5 was used.[10]

Thannhauser and Andersen Technic.—There was 0.5 c.c. of freshly prepared diazo reagent mixed with 1 c.c. of plasma, and fifteen minutes allowed for full color development. There were 2.5 c.c. of 95 per cent alcohol and 1 c.c. of saturated aqueous solution of ammonium sulphate then added. After thorough mixing and centrifuging, a portion of the supernatant fluid was removed and diluted with 33 per cent alcohol until the color matched that of the standard. In estimating the bilirubin, a dilution factor of 1 in 4 was used.*

DISCUSSION OF RESULTS

There is fairly general agreement that loss of bilirubin due to occlusion in the protein precipitate does not occur or is negligible when there is no "direct" bilirubin in the blood. In such cases, there are no significant differences between values obtained by the two methods. In this investigation, therefore, only plasmas containing "direct" bilirubin were used. All showed a "biphasic" direct reaction. In all, one hundred determinations were made in forty-three cases. The plasma bilirubin ranged between 0.3 and 32.0 units. Losses of bilirubin with the van den Bergh technic were calculated from the differences between values obtained by the two methods, it being assumed that no loss occurred with the Thannhauser and Andersen technic. The combined results are shown in Table I.

TABLE I

SHOWING LOSS OF BILIRUBIN WITH THE VAN DEN BERGH METHOD

	BILIRUBIN RANGE (UNITS)	NUMBER OF TESTS	PERCENTAGE LOSS				
			NONE	-20	21-40	41-60	61-80
Combined data	0.3-32.0	100	44	21	14	16	5
	- 4.0	66	40	8	4	10	4
	4.1-32.0	34	4	13	10	6	1
Subsiding jaundice	1.0-13.2	27	0	1	5	16	5
All cases, except subsiding jaundice	0.3-32.0	73	44	20	9	0	0
	- 4.0	51	39	9	3	0	0
	4.1-32.0	22	5	11	6	0	0

It will be observed that, with the van den Bergh technic, losses of bilirubin ranged between zero and over 60 per cent. When the data are grouped

*In 65 of the 100 determinations, the dilution factor was calculated from the volume of protein precipitate and supernatant fluid as suggested by White.[9] The differences between bilirubin values obtained by the assumed (1:4) and calculated factors were not sufficient to affect the significance of the results.

according to concentration of bilirubin, it would appear that, in general, the higher the concentration the greater the loss. Losses ranging from zero to over 60 per cent were, however, still noted regardless of concentration. In view of the observation that the van den Bergh method was found unsatisfactory in cases of subsiding jaundice, it is of interest to note that the greatest losses occurred in this type of case; of 27 such determinations, losses exceeded 40 per cent in 21. In all other cases, the loss of bilirubin with the van den Bergh technic was relatively small; though losses were greater with plasmas of high, than with those of low, bilirubin content, they never exceeded 40 per cent and, in most, were less than 20 per cent regardless of concentration. That the marked losses of bilirubin noted in the cases of subsiding jaundice were not accidental is shown by repeated determinations in the same individual (Tables II, III, and IV).

TABLE II

SHOWING A SERIES OF DETERMINATIONS IN THE SAME INDIVIDUAL

HOSPITAL NO. 3122/33. DIAGNOSIS: CATARRHAL JAUNDICE

PLASMA BILIRUBIN (UNITS)		LOSS OF BILIRUBIN WITH VAN DEN BERGH METHOD (PER CENT)	REMARKS
VAN DEN BERGH METHOD	THANNHAUSER AND ANDERSEN METHOD		
4.8	5.2	8	No skin jaundice
12.0	14.4	17	Definite skin jaundice
19.0	19.4	2	Marked jaundice
22.0	20.4	0	
15.0	16.8	11	Depth of jaundice stationary
8.0	10.4	23	Jaundice commencing to fade
5.5	9.6	43	
4.0	6.4	37	
2.0	5.3	62	Jaundice rapidly subsiding, but marked jaundice still present

TABLE III

SHOWING A SERIES OF DETERMINATIONS IN THE SAME INDIVIDUAL

HOSPITAL NO. 3251/33. DIAGNOSIS: OBSTRUCTIVE JAUNDICE—CARCINOMA OF HEAD OF PANCREAS

PLASMA BILIRUBIN (UNITS)		LOSS OF BILIRUBIN WITH VAN DEN BERGH METHOD (PER CENT)	REMARKS
VAN DEN BERGH METHOD	THANNHAUSER AND ANDERSEN METHOD		
12.0	16.8	29	
13.0	15.6	17	
14.0	14.4	3	
12.0	16.8	29	
14.0	16.8	17	Jaundice remaining stationary to this time
21.0	26.4	20	
3.5	7.2	51	Jaundice subsiding following cholecystenterostomy
1.7	3.6	53	
1.8	3.8	53	Very slight skin jaundice still present
0.8	1.8	55	No skin jaundice
0.6	1.0	40	

TABLE IV

SHOWING A SERIES OF DETERMINATIONS IN THE SAME INDIVIDUAL

HOSP. NO. 3516/33. DIAGNOSIS: CATARRHAL JAUNDICE

PLASMA BILIRUBIN (UNITS)		LOSS OF BILIRUBIN WITH VAN DEN BERGH METHOD (PER CENT)	REMARKS
VAN DEN BERGH METHOD	THANNHAUSER AND ANDERSEN METHOD		
32.0	31.2	0	
24.0	24.0	0	Depth of jaundice stationary
11.0	13.2	17	Jaundice commencing to fade
4.7	8.8	47	Jaundice rapidly subsiding
4.0	9.2	56	
1.3	4.8	73	Still slight jaundice

The observation that the van den Bergh technic is not even approximately quantitative in subsiding jaundice explains largely the absence of relationship noted at times between the clinical picture (color of the skin) and the bilirubin content of the blood in this type of case. With the Thannhauser and Andersen technic the discrepancy is not nearly so marked, although there is still evidence that, at times, the rate at which bilirubin enters the blood from the tissues is lower than the rate at which it can be removed. In all other types of cases investigated, the van den Bergh technic, although not strictly quantitative, is sufficiently so for clinical purposes. It may here be noted that it was found that in the detection of "latent" jaundice, both technics are equally sensitive.

SUMMARY

One hundred determinations were made of the bilirubin content of blood by the van den Bergh and the Thannhauser and Andersen methods in cases with "direct" bilirubin. Both methods were used in each case and determinations were made simultaneously.

In subsiding jaundice, the values obtained by the van den Bergh technic are not even approximately correct; loss of bilirubin may be as much as 60 per cent or more. In such cases, therefore, the Thannhauser and Andersen procedure is indispensable. In all other cases, however, including latent jaundice, the van den Bergh technic, although not strictly quantitative, is sufficiently so for clinical purposes.

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A METHOD OF PREPARING DUODENAL POUCHES FOR USE IN EXPERIMENTAL WORK*

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During the course of experimental investigations of the secretions found in the duodenum, it sometimes becomes desirable to prepare an isolated segment of the duodenum, one end of which opens on the abdominal wall. By means of such a pouch studies may conveniently be made of the duodenal contents, uncontaminated by juices from the stomach or jejunum. If the openings of the bile and pancreatic ducts are included in the segment of duodenum utilized for the pouch, as is usually the case, certain difficulties present themselves. As Elman and McCaughan[1] have shown, the duration of life after a fistula of the pancreatic ducts has been established is a matter of only a few days, and the animal will thus not live long enough to permit firm healing of the closed end of the pouch. During the first week there is apt to be an inflammatory reaction at the suture line which might interfere with normal experimental results, and in addition if fluid is introduced into the pouch under any degree of pressure there may be leakage at the suture line. We have had occasion to prepare and use a number of duodenal pouches,[2] and have found that the above factors are eliminated if the pouch is made by the following method:

Under intratracheal ether anesthesia, and using aseptic technic, the abdomen is opened through a midline incision extending from the ensiform cartilage downward about 4 inches. (We have used the dog in all of our work.) The first part of the jejunum is identified and marked by an Allis forcep. The pyloric end of the stomach and the first portion of the duodenum are brought up, packed off, and severed between heavy clamps at the pylorus. The end of the stomach and the adjacent end of the duodenum are then inverted and closed by two layers of 00 chromic catgut. The previously marked jejunum is then approximated to the anterior surface of the stomach and a gastroenterostomy is performed. The abdomen is closed in the usual manner. This constitutes the first stage of the operation, and the animal is now allowed a period of recuperation, usually about two weeks, before the second procedure. During this time

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the closed end of the duodenum, which will be the blind end of the pouch, has an opportunity to heal completely, and the animal returns to a normal physical condition.

The second stage of the operation requires only a few minutes and is performed as follows: The abdomen is again opened through a part of the length

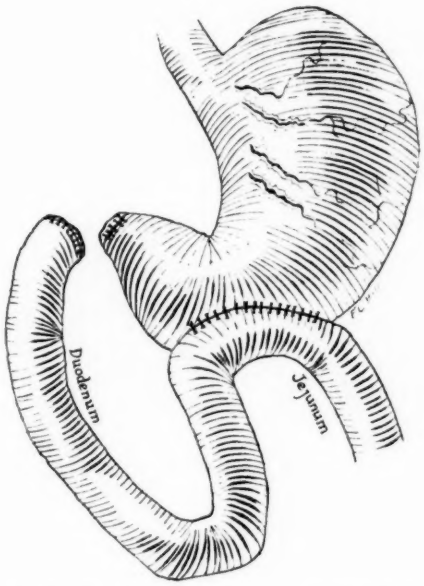

Fig. 1.—The first stage in the preparation of a duodenal pouch. The duodenum has been severed from the stomach at the pylorus, the open ends have been closed, and an anterior gastroenterostomy has been performed.

Fig. 2.—At the conclusion of the second stage of the operation; the duodenum has been cut off almost 6 inches from the pylorus and the open end has been brought through the abdominal wall. The distal duodenal stump has been closed.

of the original incision, and the duodenum is brought up into the wound. The field is packed off, and crushing clamps are placed on the duodenum about six inches from the closed end. The duodenum is severed between the clamps, and the stump nearest the jejunum is inverted and closed by two layers of

00 catgut or fine silk. A stab wound is made through the right rectus muscle near the costal margin, an Allis forceps is inserted into the abdomen through the stab wound, the crushing clamp is removed and that end of the duodenum is grasped and drawn out. The agglutination of tissues produced by the heavy clamp keeps the end of the duodenum closed while it is being drawn out, and there should be no soiling by duodenal juices. The open end of the duodenum is anchored to the peritoneum, fascia, and skin, care being taken not to interfere with the blood supply. The abdominal wound is closed again by the usual method. There is usually some shrinkage of the pouch, but we have found that if it is originally made 6 inches long, it will remain satisfactory. Most of our pouches held about 30 c.c. The pouch, when completed, retains its original nerve supply and the blood supply is, of course, very carefully preserved.

Fig. 3.—Photograph of the dog's abdomen showing the stoma of the pouch with the catheter and rubber stopper in place. Fluid is being withdrawn from the pouch by means of a syringe.

Owing to the extreme rapidity with which the physical condition of the animal deteriorates as soon as the bile and pancreatic juices begin to drain to the outside, it is advisable to start the proposed investigations within a short period of time. We are accustomed to begin the first series of experiments within eighteen hours after the completion of the operation. In order to keep the animal in good physical condition as long as possible, daily injections of 500 to 1,000 c.c. of 2 per cent sodium bicarbonate in normal saline solution should be given daily. We have found that fluid given subcutaneously in the dog is absorbed rapidly and can be given with ease and in large amounts. If the animal is in a debilitated condition, it is frequently difficult and time consuming to give fluid intravenously each day, and the subcutaneous route has given perfect satisfaction.

In a pouch prepared by the above method, with the duodenum brought out through the rectus muscle, there would in time be some valve action developed

at the stoma as the mucosa shrinks down. When the pouch is used early, however, and the mucosa still protrudes some distance from the wound, we have found it necessary in most cases to use a mechanical method of insuring that there will be no loss of fluid during an experiment. A rubber catheter, size 16 French, is passed through a rubber stopper of such size as to occlude the opening of the pouch. The catheter and stopper are adjusted so that when the stopper is in place in the stoma the catheter will project about 2 inches into the pouch. By means of a syringe, fluid may now be introduced into or withdrawn from the pouch without any leakage, especially if the stopper is held in place by manual pressure. In some cases it may be found that there is oozing of blood from the protruding mucosa in the earlier experiments, before any healing has taken place. If this might invalidate the experimental data, the bleeding may be easily stopped by touching the hemorrhagic edges with the cautery.

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RETICULOCYTES*

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RETICULOCYTES are young red cells, and therefore, a reticulocyte count gives the best evidence of the state of activity of the bone marrow. It is indicated in the study of any anemia and is especially important if pernicious anemia, aplastic anemia, or familial hemolytic icterus is being considered. An increase in the reticulocytes is the first indication of a favorable effect from therapy in anemias, whether that therapy be liver, iron, or any erythropoietic stimulant. Since the reticulocyte count is so important and is so frequently done, it is desirable to have a simple and accurate method which will give uniform results. It is evident from the large number of methods in use and from the wide variation (from as low as 0 to 0.1 per cent[1] to as high as 1.0 to 3.0 per cent[2]) in values given for normals, that a uniformly satisfactory method has not yet been devised.

While attempting to find the most desirable method of those now in use, a technic was worked out which seemed even more simple and showed more reticulocytes in a particular blood than any other method tested. It has the

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additional advantage of being adapted for use with oxalated venous blood in our uniform system[3] of hematologic methods. Evidence was further adduced to show that the reticulocyte stain is not a vital stain, as is usually stated, but will stain dead cells, if they are not fixed.

STUDY OF METHODS

Methods for reticulocyte staining may be classified in the following groups:

1. Methods in which the dye is dried on the cover slip or slide.
   (a) Moist cover slip preparations.
   (b) Dried smears.
2. Methods in which the blood is mixed with a solution of the dye.
   (a) On the slide or cover slip.
   (b) In test tube, pipette, watch glass, etc.

Obviously, the concentration, time of exposure to, and nature of the dye used may be varied with each one of these fundamental technics. Various dyes have been tried, but brilliant cresyl blue has been found so superior that the others need not be considered.

A series of experiments were performed to decide which of the above fundamental technics was most satisfactory. The methods were selected from the current literature and the textbooks in common use.

Blood was drawn from the vein of a patient with pernicious anemia and used for Methods A to F below and the remainder mixed with 2 mg. of potassium oxalate per cubic centimeter of blood and used for our experiments with oxalated blood. Five hundred red cells were counted in each experiment.

Method A: A small drop of fresh blood was placed on a cover slip, ringed with vaseline to prevent drying, and inverted onto a slide which had been smeared with a drop of saturated alcoholic solution of brilliant cresyl blue and allowed to dry. These were examined after thirty minutes. Some reticulocytes could be seen, but the smears were too thick and the red cells too unevenly distributed for accurate counting.

Method B: Smears of fresh blood were made on slides which had been smeared with a drop of saturated alcoholic solution of brilliant cresyl blue and allowed to dry. These smears gave a count of 7.6 per cent reticulocytes, which seemed to be well stained.

Method C: One drop of fresh blood was added to one drop of stain on a slide, mixed and smeared. The stain was made by mixing 2 c.c. of 1 per cent aqueous brilliant cresyl blue and 8 c.c. of 0.2 per cent potassium oxalate in 0.6 per cent sodium chloride solution, giving a final concentration of 0.2 per cent brilliant cresyl blue, 0.18 per cent potassium oxalate and 0.48 per cent sodium chloride. These smears gave a count of 8.2 per cent reticulocytes, which seemed to be well stained.

Method D: Fresh blood was drawn up to the 1 mark in a white cell pipette and filled with stain to the 11 mark and mixed thoroughly. After standing fifteen minutes, the pipette was shaken for two minutes and smears made, drying above a flame. The stain was made by mixing 3 parts of a 0.3 per cent solution of brilliant cresyl blue in 0.85 per cent sodium chloride and 1 part 0.3 per cent sodium citrate in 0.6 per cent sodium chloride, giving a final concentration of 0.075 per cent brilliant cresyl blue, 0.075 per cent sodium citrate, and 0.66 per cent sodium chloride. An occasional reticulocyte was seen, but the cells on these smears were crenated, making accurate counting impossible.

Method E: One drop of fresh blood was added to 20 drops of stain (prepared as in Method C) in a centrifuge tube, let stand for twenty minutes and centrifugated. Smears were

made of the sediment, drying over a flame. These smears gave a count of 9.6 per cent reticulocytes, which seemed to be well stained. The red cells were crenated in several of these smears.

Method F: Two drops of fresh blood were added to 25 drops of stain in a centrifuge tube, allowed to stand for twenty minutes, and then centrifugated. The stain was prepared by mixing 1 part of 1 per cent brilliant cresyl blue in 0.85 per cent sodium chloride and

TABLE I

METHOD	RETICULOCYTES
A	Poor distribution
B	7.6%
C	8.2%
D	Crenated
E	9.6%
F	17.6%
G	23.6%

5 parts of 1.0 per cent potassium oxalate in 0.85 per cent sodium chloride, giving a final concentration of 0.17 per cent brilliant cresyl blue, 0.17 per cent potassium oxalate, and 0.85 per cent sodium chloride. Smears were made of the sediment, drying over a flame. These smears gave a count of 17.6 per cent reticulocytes, which seemed to be well stained. There was a tendency toward crenation of the cells.

From these experiments, it was concluded that the fundamental Method 2(b) is much the most satisfactory because it permits more accurate control of the concentration of dye with which the cells come in contact and of the time of exposure of the cells to the dye, and because the technic is simple and gives permanent preparations if counterstained with Wright's stain. The wide variation in counts on the same blood specimen by the different methods (Table I) proved that accurate control of these factors is necessary, and that most, if not all, of these methods fail to demonstrate all the reticulocytes actually present.

Further experiments were planned to find out whether oxalated blood could be used and to determine the optimum concentration of dye and time of exposure. Using Method F, which gave the highest count with the fresh blood (17.6 per cent) and the oxalated blood which had stood at room temperature for one hour, a count of 14.6 per cent reticulocytes was obtained, proving oxalated blood to be satisfactory. By using equal parts of oxalated blood and dye solution, satisfactory smears were obtained without centrifugation. Since the blood already contained an anticoagulant, the oxalate solution was omitted and mixing the blood with an equal volume of brilliant cresyl blue solution in 0.85 per cent sodium chloride was tried. Using a series of dye concentrations from 0.075 per cent to 14.0 per cent (saturated), it was found that a 1.0 per cent solution gave the maximum number (23.6 per cent) of reticulocytes with the minimum amount of débris on the slide. Concentrations below 1.0 or over 3.0 per cent gave low counts; the concentrations above 1.0 up to 3.0 per cent gave quite satisfactory smears when counterstained with Wright's stain. Using equal parts of the 1.0 per cent dye solution and oxalated blood, the time of exposure to the dye was varied from mixing on the slide (fifteen seconds)

to twenty-four hours, and it was found that results were satisfactory for all times over one minute, but that low counts were obtained if the time were thirty seconds or less. Red cell counts made on the original blood and on an accurate dilution with the dye solution showed that no hemolysis occurred.

From these experiments, it was concluded that the following is the most satisfactory technic for staining and counting reticulocytes: Mix, in a small test tube, equal parts (5 drops) of oxalated venous or capillary blood and 1 per cent of brilliant cresyl blue in 0.85 per cent sodium chloride solution. Let stand at least one minute, mix, and make thin smears, drying in the air as usual. These smears may be counted at any time within twenty-four hours, but if a permanent preparation is desired, they should be counterstained with Wright's stain by the usual technic. The brilliant cresyl blue solution keeps well but should be filtered if débris appears on the slide. Select an area on the slide which contains 50 to 75 red cells per oil immersion field. Count all the cells and all the reticulocytes in as many adjacent fields as is necessary to give a total of 500 red cells if the count is over 5 per cent, or 1,000 red cells if the count is less than 5 per cent reticulocytes. With this method, a count of 23.6 per cent was obtained in the blood which gave 17.6 per cent with the best method previously studied.

Using this technic (Method G) reticulocyte counts were made at intervals for twenty-four hours after drawing blood, and then at twenty-four-hour intervals up to ninety-six hours, and again at one hundred and forty-four hours. To our surprise, the counts showed no significant variations during the six days (Table II), although after ninety-six hours the blood was badly hemo-

TABLE II

TIME AFTER DRAWING BLOOD	RETICULOCYTES PER CENT
1 hr., 30 min.	22.6
3 hr.	19.2
5 hr.	27.6
23 hr.	22.8
24 hr.	23.2
48 hr.	27.0
72 hr. (some hemolysis)	23.8
96 hr. (marked hemolysis)	26.8
144 hr. (marked hemolysis)	28.0
Average	23.6

TABLE III

TIME	OXALATED BLOOD RETICULOCYTES PER CENT	OXALATE + FLUORIDE RETICULOCYTES PER CENT
	11.40	
5 min.		10.20
30 min.	10.80	8.85
2 hr.	8.00	10.80
3 hr.	10.80	12.00
26 hr.	12.20	8.80
Average	10.64	10.13

TABLE IV

RETICULOCYTES PER CENT	RETICULOCYTES PER C. MM.		RETICULOCYTES PER CENT	RETICULOCYTES PER C. MM.
Men			Men	
0.5	26,050		1.4	74,620
0.6	28,320		1.5	65,850
0.6	30,120		1.5	72,300
0.6	32,220		1.5	81,450
0.6	34,620		1.5	81,900
0.6	36,180		1.5	82,200
0.7	29,890		1.5	92,850
0.7	33,600		1.6	73,760
0.7	33,600		1.6	82,880
0.7	34,720		1.6	86,400
0.7	35,730		1.6	86,560
0.7	35,770		1.6	87,360
0.7	37,100		1.7	80,920
0.7	37,170		1.7	93,670
0.7	37,940		1.7	100,810
0.7	40,250		1.8	97,200
0.8	32,960		1.8	98,460
0.8	37,040		1.8	107,460
0.8	41,120		1.8	107,640
0.8	43,360		1.8	108,180
0.8	43,680		1.8	111,780
0.8	50,800		1.9	95,190
0.9	38,970		1.9	102,600
0.9	40,850		1.9	103,550
0.9	44,730		2.0	98,000
0.9	45,090		2.0	106,200
0.9	46,620		2.0	107,600
0.9	47,610		2.0	116,600
0.9	49,680		2.1	104,580
0.9	49,950		2.1	106,890
0.9	52,200		2.2	104,940
1.0	50,200		2.2	111,100
1.0	51,500		2.2	111,760
1.0	51,900		2.3	114,770
1.0	52,100		2.3	115,230
1.0	52,900		2.3	123,970
1.1	52,140		2.4	120,000
1.1	54,000		2.4	126,720
1.1	55,440		2.5	140,000
1.1	56,100		2.6	136,240
1.1	58,300		2.6	156,520
1.1	58,850		2.8	137,200
1.1	59,070		2.9	144,130
1.1	61,490		2.9	175,450
1.2	53,160		2.9	195,870
1.2	53,880		3.0	133,200
1.2	55,800		3.0	184,500
1.2	58,680		3.1	148,800
1.2	61,560		3.2	171,200
1.2	62,640		3.3	165,660
1.2	67,400		3.4	172,720
1.3	70,200		3.4	201,620
1.3	70,850		3.8	211,660
1.3	77,090			
1.3	78,910	Average	1.57	83,160
1.4	70,560	Minimum	0.50	26,050
1.4	70,700	Maximum	3.80	211,660

TABLE IV—CONT'D

RETICULOCYTES PER CENT	RETICULOCYTES PER C. MM.		RETICULOCYTES PER CENT	RETICULOCYTES PER C. MM.
Women			Women	
0.5	21,400		1.5	75,300
0.5	22,300		1.6	65,280
0.8	37,120		1.6	72,800
0.9	36,450		1.6	73,600
0.9	41,040		1.6	76,000
0.9	41,580		1.6	82,240
0.9	42,930		1.8	71,100
0.9	44,640		1.8	79,200
0.9	45,090		1.8	84,620
1.0	46,300		1.8	88,200
1.0	46,500		1.9	92,950
1.0	47,000		2.0	91,400
1.0	49,600		2.1	106,470
1.1	44,000		2.2	96,360
1.1	49,390		2.2	100,320
1.1	49,610		2.2	107,800
1.1	50,710		2.3	103,960
1.2	53,160		2.5	121,500
1.2	56,880		2.6	120,120
1.2	60,120		2.6	131,300
1.3	51,740		3.3	150,150
1.4	51,180		3.5	156,800
1.4	69,860		3.6	153,360
1.4	72,800			
1.5	63,200	Average	1.57	72,800
1.5	66,900	Minimum	0.50	21,400
1.5	67,950	Maximum	3.60	156,800

lyzed. This showed that oxalated venous blood could be safely used for at least twenty-four hours, and suggested to us that this was not a vital stain, if the term "vital stain" is used in its strictest sense of a stain which acts only on living cells and in staining causes cell death. To aid in deciding this point, equal parts of oxalated blood and 1 per cent sodium cyanide were mixed and reticulocyte counts were done at intervals up to three hours. No significant variations in the reticulocyte counts from those on the untreated blood were found. Another sample of oxalated blood was mixed and divided into two parts. To one part sodium fluoride was added in the proportion of 10 mg. per c.c. Reticulocyte counts were made at intervals for twenty-four hours on both samples with the results shown in Table III. When the red cells were fixed by methyl or ethyl alcohol, formalin, or flame, the reticulocytes did not stain. The results of these experiments support the opinion[4] that the reticulum can be stained in unfixed, but not necessarily living, red cells.

NORMAL VALUES

To determine normal values for the improved technic, reticulocyte and red cell counts were done on 110 healthy young men and 50 healthy young women, with the results shown in Table IV. Note that the average figure is 1.57 per cent and the range from 0.5 to 3.8 per cent for both men and women. The total number of reticulocytes calculated from the red cell count averages 83,160 per c.mm. for men and 72,800 per c.mm. for women and shows a range of from

26,050 to 211,660 and from 21,400 to 156,800, respectively. Studies[5] of normal hematology of over 200 children of school age show practically the same percentage values for reticulocytes. For clinical purposes, the average normal may be regarded as 1.5 per cent with figures below 0.5 or above 4.0 per cent, indicating decreased or increased red cell formation, respectively. Observe that these figures are significantly higher than those generally given and that reticulocytes were found in all normal bloods.

SUMMARY

A study of the methods for reticulocyte staining in common use showed a great number of different technics giving widely variant results.

A simple technic was worked out which gives uniformly good results and significantly higher counts than other methods tested.

Normal values for this method are 1.5 per cent with a range of 0.5 to 4.0 per cent. Since the percentage values for both sexes at all ages are the same, while the absolute counts differ markedly, it would seem better to report reticulocyte counts in percentage rather than in absolute numbers.

Evidence is presented which suggests that the term "vital" should not be used when referring to the reticulocyte stain.

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SMALL ANIMAL METABOLISM CAGE*

Seward E. Owen, Ph.D., Hines, Ill.

THE cage design presented here offers several advantages over the usual small animal cage. Among these advantages are (1) low cost per cage, the size given when completed averaging about forty cents each; (2) ease of construction, as no solder is necessary; (3) ease of cleaning; (4) immediate accessibility to the animals, which are visible at all times; (5) feed boxes and water founts which can be changed without opening or moving the cage; (6) the construction which

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permits of separation of urine and feces for examination; (7) the convenience of weighing animals without removing them from the cage.

The construction requires no special tools other than a pair of heavy shears, or tin snips, and pliers. The cage proper is a cylinder of ⅝-inch mesh galvanized screen with a fitted bottom of the same material. Covers are made from 8½-in. tin pot covers; the bottoms are tin pie pans of the same dimension. These items can readily be obtained from the local stores.

The cage size as used in our laboratories for rats is 10 in. high and 8 in. in diameter with the floor placed about 2½ in. from the cylinder bottoms. This height prevents coprophagy in vitamin work.

The mesh is cut so as to leave protruding mesh ends which are bent to form an L. These are clinched over in forming the cylinder as shown in Fig. 2, *I*. The floor of the cage may be wired to the walls as at *x* (Fig. 1), or stiff cross-

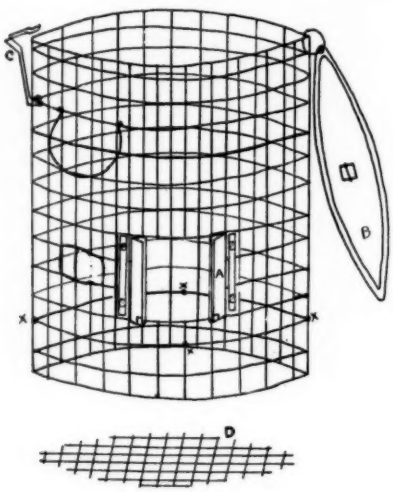

Fig. 1.

wires such as made from baling wire may be placed across the inside of the cylinder for the bottom to rest on. This latter procedure may allow of easier cleaning although some rigidity is lost by it. If the floor is made removable as suggested, it should be bound with tin strips. Such strips can be wired to the floor plate easily by punching holes in the binding strip. The cover hinge as well as the snap and water-fount support are made from baling wire. The cover hinge is an overlapped ring of the wire placed as shown.

The food tunnel is shaped from material obtained from discarded tin cans. These pieces were made into boxes about 10 cm. long by 5 cm. square with one end closed and the edges of the open end flared to fit the slots attached to the cage at *A* (Fig. 1). Small aluminum rivets were used to hold the box rigid and to attach the top of an ointment jar. The ointment jars or food jars are of porcelain or glass 6.5 cm. in diameter and 6 cm. high. By cutting an opening through the bottom of the feed tunnel and the attached ointment jar top, the animal is allowed ready access to the food. Spillage and wastage is thus cut

down, and the food jar may easily be removed for weighing, cleaning, and filling. If desired, the food tunnel opening can be fitted with tin squares which have holes of various sizes according to the rat size. In other experiments not requiring the accurate checking of food consumption, the slot *A* holds a plain piece of sheet tin and the food jars may be placed inside the cage. For daily checking of food weights, a glass of food or seed jar such as is used on canary cages may be used; this makes a more compact assembly, but these jars do not hold sufficient food for several days' feeding.

The water founts are the usual inverted bottles with L-shaped glass tubes having an opening blown on the upper surface of the lower limb as sketched at *H* (Fig. 2).

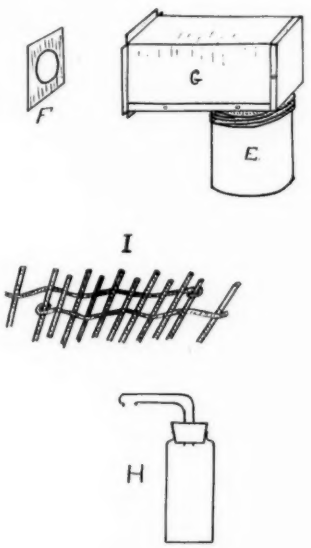

Fig. 2.

When using the cages in which separate urine and feces samples were desired, six-inch holes were bored in the shelf supporting the cages. Seven-and-a-quarter-inch glass funnels were placed in these holes and the cages placed over the funnels. Screens made from No. 60 brass strainer net were placed in the funnels to collect feces while the urine drained into small tubes or flasks placed at the funnel tips on a lower shelf. The funnel tip may be narrowed to deliver drops and a continuous record of urine secretion obtained by the use of methods suggested by Owen.[1] While we have routinely filtered urines before extraction in studies of drug excretion (Owen and Olsen[2]), this is usually not essential unless a dry, finely divided food is used.

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DEPARTMENT OF REVIEWS AND ABSTRACTS

ROBERT A. KILDUFFE, M.D., ABSTRACT EDITOR

TUBERCULIN, Ointment Patch Test, Wolff, E. Am. J. Dis. Child. 47: 764, 1934.

The author describes tests with an ointment composed of highly concentrated tuberculin and killed tubercle bacilli. The area of application is the paravertebral region between the eighth and eleventh thoracic vertebrae, or the medial brachial surface.

The skin is cleansed with green soap and water, dried gently, bathed with benzene, and then gently dried again. The application of benzene only, as heretofore practiced, is not sufficient to remove the secretions from very oily skins. A pea-sized drop of the tuberculin ointment is applied on the right side, and a similar sized drop of the control ointment is applied on the left side. Each of these drops is covered with a 2-inch (5 cm.) square of adhesive tape.

The test is read in forty-eight hours. The adhesive tape is removed after soaking with benzene, and the area is gently cleansed with benzene. The reaction may be clearly observed ten minutes after the removal of the tape.

Positive reactions exhibit papules, erythema, induration, and pigmentation. A weak positive test shows a few (not more than twenty) discrete papules from 1 to 3 mm. in diameter and pale rose in color. A medium reaction shows many vividly red papules surrounded by the normal skin. A strong reaction shows, in addition, changes of the total test area consisting in yellow brown pigmentation, indurative formation of plateaus, and marked reddening. A few or all of the papules may even show distinct formation of vesicles.

In weak reactions it is advisable to palpate the area in order to detect the follicles and to compare it with the area of the control.

The previously mentioned yellow pigmentation of the entire area is easily brought out by stretching the skin, whereas stretching the control area only blanches it.

The peak is reached after seventy-two hours, regardless of whether the test area was covered with adhesive tape for forty-eight or for seventy-two hours. This shows definitely that the reactive substances are absorbed by the skin within forty-eight hours and that the reaction of the skin to the absorbed material continues after the removal of the tape.

The color of the reaction begins to fade within ninety-six hours and is still faintly discernible up to one week. Medium reactions show a definite scaling up to approximately ten days; and in the marked reaction, even after two weeks, there may be roughening of the skin, brownish discolorations, infiltration, and scaling of the area.

In contradistinction to the intracutaneous test, there has never, thus far, been a generalized systemic reaction such as a rise in temperature, malaise, or focal signs.

Subjective symptoms accompanying the local reactions are mild. In nearly every positive case there is some itching over the test area, with only slight discomfort. In rare cases of very marked sensitiveness, slight pain over the test area may occur.

The ointment is stable and easier to apply, and it causes less psychic disturbance and fewer untoward general and focal reactions in the patient than does the old tuberculin used in the intracutaneous test.

The agreement between the ointment patch test and the intracutaneous test with tuberculin in all dilutions as performed on 190 children was 95.8 per cent.

The ointment patch test is generally comparable in results with an intracutaneous injection of 0.1 c.c. of old tuberculin, 1:100.

The ointment patch test produced markedly less nonspecific cutaneous irritation than the intracutaneous test.

TUMOR GROWTH, "Heavy Water" and, Woglom, W. H., and Weber, L. A. J. A. M. A. 102: 1289, 1934.

Deuterium, in the amounts that it was possible to administer as "heavy water," had no demonstrable effect on the growth of mouse sarcoma or mouse carcinoma.

TETANUS: Development of Tetanus Antitoxin Following Administration of Tetanus Toxoid, Sneath, P. A. T. J. A. M. A. 102: 1288, 1934.

Of 29 persons given three doses of tetanus toxoid, significant amounts of antitoxin developed in 28, a titer of at least 0.1 unit per cubic centimeter being reached in the majority, or 20.

From five to seven months after the last dose there was, in general, a reduction in the antitoxin level, but 27 still showed demonstrable antitoxin; and 25 showed 0.01 unit or more.

This suggests that active immunization with tetanus toxoid might be adopted advantageously by certain groups in whom the hazard of tetanus is greater than in the general population.

MENINGITIS, Pneumococcic, Report of Case With Recovery Following Cisternal Drainage, Bedell, C. C. J. A. M. A. 102: 820, 1934.

In a case of pneumococcic meningitis, Group IV, a drainage tube was inserted into the cisterna magna as soon as signs of meningeal invasion were observed. The tube remained in place for eight days; 200 c.c. of cerebrospinal fluid escaped daily by this route. Irrigations with physiologic solution of sodium chloride were carried out from the cisterna magna to the lumbar region. Periodic shrinkage of the brain volume was attempted by intravenous injections of hypertonic dextrose. The spinal fluid became secondarily infected with *Bacillus proteus* at the end of six days, producing a marked leucocytic response. There was progressive improvement, however, and the patient recovered completely and left the hospital at the end of seven weeks.

GONOCOCCI, Preparation of Correct Picture and Simple Culture Method, Szilvasi, J. Dermat. Wchnschr. Leipzig, 97: 1811, 1933.

To obtain the material for microscopic examination Szilvasi lets the patient urinate and then inserts a platinum loop into the anterior portion of the urethra and obtains a secretion that is readily spread out. After staining according to Gram's method, a striking picture is seen. Between monocytes and isolated threads of mucus, the gonococci are found in peculiar arrangements, in the form of coils, of clusters, or of wreaths or caps. The fact that an intensely stained zone surrounds the individual gonococcus enhances the peculiarity of the picture.

This method of obtaining the specimen of the urethral secretion is advantageous also for the culture of the gonococcus, a procedure which used to be rather difficult when the thick pus was used. The secretion is applied to the culture medium not in streaks, but in dots, and the gonococci are found on the margin of the dots. The facts that in the pus the majority of gonococci are either destroyed or impaired and that the phagocytic leucocytes contain other microorganisms are probably the reasons why the demonstration and culture are less successful when thick pus is used than when the secretion is used.

ANEMIA, Aplastic, Greenwald, H. M. Am. J. Dis. Child. 47: 360, 1934.

It is now generally conceded that aplastic anemia is a disease *sui generis* and is not a biologic variation of some form of primary or secondary anemia.

The most characteristic pathologic finding is a sulphur yellow appearance of the bone marrow instead of the reddish appearance one would expect to find in severe anemia. This is

due to a replacement of the normal marrow by ordinary fat tissue. In the case reported, the bone marrow was of a cranberry red appearance. Histologic examination showed, however, that the reddish appearance of the bone marrow was due not to active regenerative processes, but to hemorrhage. Histologic examination revealed a total absence of nucleated red blood cells and myeloid elements.

The blood picture is, of course, the main point of clinical diagnosis. There is a marked decrease in the hemoglobin content, with a diminution in the red blood cells to 1,000,000 or less; the hemoglobin is reduced to 10 or 15 per cent. The color index is approximately 1. Nucleated red blood cells are usually absent although occasionally observers have found isolated normoblasts. Reticulocytes and polychromatophilic erythrocytes are absent. There is leucopenia with a marked reduction of the neutrophilic leucocytes and an almost complete absence of eosinophils. There is a relative increase in the lymphocytes. The differential count often shows the presence of only from 8 to 10 per cent polymorphonuclear cells while the lymphocytes are frequently found to be 90 per cent or over. Thrombocytopenia is constant and is a feature of the disease. The coagulation time is normal, but the retraction of the clot is interfered with, and the bleeding time is prolonged. In addition to the absence of normoblasts and megaloblasts, myelocytes, myeloblasts, or other forms of embryonic cells are usually absent. The results of the fragility test are usually normal; Lucas, however, stated that the fragility of the red cells may be altered, owing to the inability of the bone marrow to produce new red cells.

The blood serum is water clear. There is no increase in bile pigments. Urobilinuria is absent, and there is no increase in the urobilin content of the stool.

The course of the disease is rapid, and it is usually fatal before four months have elapsed. In fact, some observers refuse to regard the case of a patient who lives longer than six months as a true case of aplastic anemia.

Up to the present no therapeutic measure has proved satisfactory in the treatment of this disease. Repeated transfusion is generally recommended, but it is, at best, only a palliative measure.

BLOOD SEDIMENTATION: The Interrelation of Cutler, Linzenmeier and Westergren Tests, Greisheimer, E. M., Treloar, A. E., and Ryan, M. Am. J. M. Sc. **187:** 213, 1934.

Blood sedimentation in 99 men and 102 women, selected without regard to age or health from university students and ambulatory patients, has been studied to establish the interrelationships between the Linzenmeier, Cutler, and Westergren methods. The average sedimentation in one hour for "normal" subjects appears to be reasonably concordant for the three methods despite the wide differences in tube width, anticoagulant concentration and length of fluid column, although the differences between the means for the three methods are significant statistically. The average sedimentation at one hour for the women is approximately double that for the men.

The concordance between the results by each pair of methods for the individual patients has been subjected to analysis by preparing the correlation tables. The regression lines, for predicting the most likely value to be expected by any one test when that by another is known, proved to be of three distinct types. There is a rectilinear relationship between the Cutler index and the Linzenmeier index. The regressions between Westergren index and Cutler or Linzenmeier index appear to be somewhat like saturation curves. However, they had to be fitted by a systematic scheme of free-hand graduations for lack of a suitable type of mathematical equation. The relationships between the various indices and Linzenmeier time values may be suitably portrayed by section of hyperbolas. Tables are provided for these average predictions for each independent variable of clinical interest.

The dispersals of individual cases about the lines of average relationship, although not studied in detail, are clearly greater than those ascribable to errors inherent in the technics.

The conclusion is clear that sedimentation measures for human blood are, in part, specific for the technic employed.

Standardization of a generally acceptable method for blood sedimentation would prove most advantageous for clinical work.

TUBERCULIN TEST: Value of the Negative Intracutaneous (Mantoux) Test in Adults, Lichtenstein, M. R. Am. Rev. Tubere. **39**: 190, 1934.

Complete insensitiveness to tuberculoprotein makes it certain that the patient is nontuberculous, with the few exceptions given.

Complete insensitiveness is found in a sufficient number of adults to make the test worth while.

Patients who react only to the strong concentrations of tuberculoprotein, with the same exceptions, almost certainly have no *active* tuberculosis.

The test quickly excludes as nontuberculous a number of suspects, including some of the most difficult cases for diagnosis.

The intracutaneous test, carried into the stronger concentrations, should be a part of the diagnostic routine of every physician considering tuberculosis.

PNEUMONIA, in Newborn and Stillborn Infants, Warwick, M. Am. J. M. Sc. **187**: 253, 1934.

Pneumonia in newborn infants is a definite entity and should be recognized by obstetricians, pediatricians, and pathologists. It usually begins in utero or during birth. The majority of the victims have physical handicaps; consequently, the pneumonia may be the result of lowered resistance. The exciting cause is as yet uncertain but seems to rest between bacteria contaminating the amniotic fluid or an irritating amniotic fluid, particularly when it contains large amounts of bile and cornified epithelial cells. Present evidence seems to favor the latter.

The facts favoring bacteria as the cause of pneumonia in the newborn infant are given:

1. Bacteria are usually the cause of exudation of polymorphonuclear leucocytes and of pneumonia.
2. Bacteria can be demonstrated in some of the pneumonic lungs.
3. In some, possible sources of infection are found, such as premature rupture of the membranes or light respiratory infections in the mother.
4. Some of the cases of pneumonia occur in groups during the months in which respiratory infections are the most prevalent.
5. Amniotic fluid is very frequently found in lungs where there is no evidence of pneumonia.

On the other hand, equally good reasons may be found for the possibility of uninfected amniotic fluid being the etiologic factor and these are also given below:

1. Chemicals may cause an exudative inflammation and also pneumonia.
2. A large percentage of cases show no demonstrable bacteria.
3. Masses of aspirated amniotic fluid are often found surrounded by numerous polymorphonuclear leucocytes in the midst of an atelectatic lung.
4. The pneumonia is often associated with atelectasis.
5. Amniotic fluid may vary markedly in constituents and in irritability.
6. Infants with other physical handicaps are often affected.
7. In some cases no convincing possibility of entrance of bacteria is seen.

TULAREMIA, Treated by a New Specific Antiserum, Foshay, L. Am. J. M. Sc. **187**: 235, 1934.

An initial trial has shown that the goat can be made to yield a potent antitularense serum which is effective in treating tularemia in man. The intravenous administration of this serum produced a marked and prompt amelioration of symptoms in 14 out of 15

tularemia patients and caused a shortening of the duration of adenopathy, the period of disability, and the total duration of disease. One patient, received in dying condition with extensive involvement of the lungs, liver, and spleen, was not improved. The antiserum has a specific desensitizing action. There is evidence to show that its beneficial effects in man are intimately associated with, and perhaps dependent upon, this property.

ARTHRITIS, Rheumatoid, Blair, J. E., and Hallman, F. A. Arch. Int. Med. 53: 87, 1934.

In a series of 57 cultures of synovial fluids and tissues from chronic multiple arthritis (55 from rheumatoid arthritis and 2 from Still's disease), a total of 41 (74.5 per cent) remained sterile and 14 (25.4 per cent) yielded positive cultures. The microorganisms obtained in the positive cultures included streptococci, diphtheroid bacilli, gram-positive cocci incapable of growth on subculture, and an occasional gram-negative bacillus associated with the aforementioned bacteria.

In a series of 175 cultures of synovial fluids and tissues from a variety of chronic infectious and noninfectious conditions of the joint other than rheumatoid arthritis, 142 (81.1 per cent) were sterile and 33 (18.8 per cent) were positive. The positive cultures included *Staphylococcus aureus*, indifferent streptococci, gram-positive cocci incapable of growth on subculture, and diphtheroid bacilli.

No direct etiologic significance is attached to any of the organisms obtained in this series.

It is felt that at the present time no specific bacteriologic agent may be considered to have been demonstrated as the etiologic cause of rheumatoid arthritis, particularly in view of the multiplicity of results and the lack of general confirmation of any one report.

BLOOD, in Normal Pregnancy, Dieckmann, W. J., and Wegner, C. R. Arch. Int. Med. 53: 71, 1934.

Previously reported values for blood and plasma volumes in pregnancy are at variance with each other, and the results are inconclusive because of the different methods used and the method of calculation. The volumes are reported either in cubic centimeters per kilogram or in percentage of body weight, either of which is unreliable because of the constantly changing weight in pregnancy.

Determinations of the blood and plasma volumes were made on various groups of women for the different periods of pregnancy. The cubic centimeters per kilogram and the means for the different periods were calculated, and although there is a slight increase at term, statistical analysis indicates that the changes are of no significance.

Similar studies in which the same women were followed throughout pregnancy and the puerperium indicate that the following changes occur:

1. The blood and plasma volumes begin to increase in the first trimester, and by the thirteenth week the gain amounts to 16 per cent and 18 per cent, respectively.

2. At term the average increase in the blood volume is 23 per cent and the plasma 25 per cent. This change is designated an "oligocythemic hypervolemia." Although this increase seems large, losses of blood of 700 c.c. or more are at once manifested in measurable reductions in volume. The pregnant woman survives losses of blood which would be fatal if she were not pregnant, partly because of the increase in blood volume, but more particularly because of the tremendous amount of fluid in her tissues.

3. At eight weeks postpartum there is an average decrease of 16 per cent for both blood and plasma volumes. This does not quite equal the increase, but, since most of the patients weigh more after pregnancy, the discrepancy is explained.

4. The increase in blood and plasma volumes is not merely to fill vessels but is probably a part of the mechanism required to permit proper fetal respiration.

PNEUMONIA, Epidemiology of Lobar, Smillie, W. G., and Leeder, F. S. Am. J. Pub. Health 24: 129, 1934.

Nasopharyngeal cultures from 264 contacts of 64 cases of lobar pneumonia due to Type I and Type II pneumococcus have been studied. The results indicate that 20 per cent of the immediate family contacts of these patients harbored the homologous strain in their nasopharynges. The hospital contacts of the same patients, however, were seldom infected (about 2 per cent). These results suggest that it is quite justifiable to treat cases of lobar pneumonia due to Type I and Type II pneumococci in the open wards of general hospitals.

One experiment indicates that a healthy carrier of Type I pneumococcus does not transmit this organism to immediate contacts, even though the individuals are living under overcrowded conditions.

The evidence suggests that there is some additional factor other than simple contact which determines the transfer of Type I or II pneumococci from a patient with lobar pneumonia to contacts. Nineteen of the 25 family contacts of cases of lobar pneumonia due to Type I or Type II pneumococci that became homologous carriers were suffering from acute colds at the time the cultures were taken. Positive cultures were found as frequently on the first day of exposure as after a week. This evidence suggests that family epidemics of colds may be a factor which determines the transfer and establishment of Type I and Type II pneumococci from the infected to the uninfected.

Carriers of Type I and Type II pneumococci, when once established, may continue as carriers of these strains for a considerable period of time without giving rise to lobar pneumonia in themselves or their contacts and without producing a second group of carriers.

MILK, Examination of for Members of the Escherichia-Aerobacter Group, McCrady, M. H., and Archambault, J. Am. J. Pub. Health 24: 122, 1934.

Comparative results obtained from examination of milk samples for Escherichia-Aerobacter by various methods suggest the following tentative conclusions:

1. So-called "typical" colonies appearing in solid media pour-plates inoculated with milk may not be accepted as colonies of Escherichia-Aerobacter unless a sufficiently thorough examination of a large number of colonies from representative samples has proved that they may be so accepted. In the hands of the authors, plates poured with neither Endo, bile salt, nor eosin methylene blue agar proved reliable for direct enumeration of colon organisms in milk samples since the proportion of resulting typical colonies which were confirmed varied from zero in some samples to 100 per cent in others. It is quite probable that similar difficulties would be encountered in the examination of dairy products other than milk by these plate methods.

2. The production of 10 per cent or more gas in brilliant green bile 2 per cent special fermentation tubes inoculated with quantities of raw or pasteurized milk varying from 10 c.c. to 0.001 c.c. and incubated forty-eight hours at 37° C. provides reasonable assurance of the presence of Escherichia-Aerobacter organisms. This medium appears to be as reliable as gentian violet bile for the enumeration of these organisms and is more conveniently employed in those laboratories where it is also used for examination of water samples. The percentage of such brilliant green bile presumptives which were completely confirmed varied as follows:

Raw milk, 1 c.c. to 0.001 c.c.	99 per cent
Pasteurized milk, 10 c.c.	100 per cent
Pasteurized milk, 1 c.c. to 0.001 c.c.	86 per cent

It is pointed out that since the organisms in 10 c.c. of properly pasteurized milk rarely produce gas in this medium, the production of any amount of gas from the majority of such samples of 10 c.c. or less of a pasteurized milk is usually indicative of some fault in the processing of the milk, either improper pasteurization or recontamination of the pasteurized product.

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EDITORIAL

Developments in the Study of Agglutinins

THE bacteriologist of the future will, without doubt, look back with envy upon the days when dogma was supreme, when bacterial species were immutable, and any variation from the accepted type was dismissed with complacence as an "involution form."

Indeed, it is quite possible that this attitude may even be encountered among the bacteriologists of today, confronted as they are with a confused array of "rough" and "smooth" colonies with H's and O's thrown in for good measure!

At first regarded as of purely academic interest, later studies have shown quite definitely that these bacterial variations have a very definite and perhaps wide application to the practical problems of bacteriology, immunity, and epidemiology and cannot be disregarded in the utilization of agglutination reactions in the study of disease.

Among the oldest and perhaps also the most commonly applied agglutination test is that devised by Widal for the study of typhoid fever; certainly it is perhaps the one most familiar to the practitioner at large and because of the fact that large numbers of the general population have now a history of antityphoid vaccination as an aftermath of the World War, general concepts as to the clinical interpretation of positive Widal reactions have, perforce, been the subject of renewed study and modification which it may be of some interest to summarize and review.

The occurrence of agglutinins for the typhoid group, as well as for other organisms, in nonvaccinated, supposedly normal individuals has been studied by numerous investigators interested, not only in their occurrence but in the explanation of their presence.

Perhaps the majority of observations in this field support the view that the occurrence of such agglutinins is the result of previous exposure to the specific infectious agent, either through vaccination or as a result of natural clinical or subclinical infection.

Havens and Mayfield[1] in a study of 1,136 such individuals encountered 263 positive reactions or 23 per cent, 14 per cent in a dilution of 1:40; 6 per cent in a dilution of 1:80, and 3 per cent in a dilution of 1:160, results comparable to those of other and previous investigators.

These so-called "normal" agglutinins behaved in the same manner and were in all respects indistinguishable from specific antibodies. As in only 60 of the 263 positive reactors was it possible to obtain any information concerning any previous attack of clinical typhoid fever or of prior vaccination, 48 having no such history, it must be assumed that, if these 60 may be taken as a fair sample of the group, only 20 per cent of all positive Widal reactions represent the aftermath of vaccination or clinical typhoid fever.

Other observers, however, have reported the apparent nonspecific production of typhoid agglutinins. For example, Kilduffe and Hersohn[2] demonstrated the presence of typhoid agglutinins in 29 per cent of 200 serums from tuberculous patients, only 33 per cent of those reacting having any history of previous vaccination or clinical typhoid fever.

In the interpretation of such reactions various possibilities must be taken into account: They may be either nonspecific or heterologous; they may represent the result of an unrecognized or subclinical typhoid infection in the past and, as such, an expression of the reawakening of a previous specific antigenic stimulus, or they may exemplify, as indicated by the observation of Gilbert and Coleman,[3] the fact that exposure to certain bacterial types will again initiate production of apparently unrelated antibodies such as have developed previously in response to an antigenic stimulus of different nature.

While there may be room for discussion as to the nature and mechanism of such reactions, there can be no question that they may definitely complicate the interpretation of diagnostic agglutination tests.

In view of the findings above referred to it is, or should be, obvious that the *trend* of a series of agglutination reactions furnishes evidence much more clear-cut and definite than that obtainable from a single test, just as the trend

of a series of leucocyte counts is of far greater value than the results of a single determination. Nevertheless, in by far the greater majority of instances, clinical investigations do not include such serial studies and clinical inferences, and deductions must stand or fall upon the results of a single agglutination test.

Under these circumstances it is fortunate that studies in bacterial mutation and variation have demonstrated a relation between this and the response of the organisms to agglutinins and that evidence is accumulating which suggests that even a single agglutination test may furnish reliable information when interpreted in the light of these newer investigations.

That variations may occur in colonies of a pure culture of a single bacterial species was long ago recorded, but it is only within comparatively recent times that it has been appreciated that these variations are indicative of variations in antigenic structure and of variation in the production and the response to agglutinins.

The starting point of these investigations may be said to be the work of Weil and Felix[4] who first demonstrated the existence of dual antigens in many bacteria and showed their relation to variations in the character of their colonies, for while the observation had been recorded before by the English workers Smith and Reagh and Beyer and Reagh, it passed almost unnoticed until the work of Weil and Felix in 1917 and their later publication in 1924 demonstrated its practical importance in the interpretation of agglutination reactions.

During the study of the anomalous Weil-Felix reaction (the agglutination of a particular strain, Proteus X19, of proteus organisms by the blood of patients suffering from typhus fever), these workers recorded first, that there occurred two types of colonies in the proteus cultures; second, that these two types presented differing agglutinogenic properties, and, finally, that the clinical interpretation of agglutination reactions was affected by and related to these differences.

The normal, flagellated proteus bacillus produces a thin, spreading growth spoken of as the "exhalation" (Hauch) form, while the nonflagellated variant presented isolated colonies without exhalation (Ohne Hauch), terms soon shortened to O and H.

Later developments demonstrated that the agglutinogenic properties of these O and H forms were different, the antigens—"H antigens" produced by the H forms being flagellar in origin and thermolabile while those produced by the O forms were thermostabile and of somatic derivation.

The subsequent studies of numerous investigators showed that dual antigens were present in many bacteria among them the organisms of the typhoid group.

The colony variation in bacteria of this and other groups not being of the same character as those seen in the proteus group, are spoken of as "smooth" and "rough" abbreviated, for convenience, to "S" and "R."

As yet, however, there is lacking any clear definition of the exact meaning of "smooth" and "rough" as applied to bacterial colonies so that, in the interests of exact terminology is has been suggested that they be replaced by "matt" and "glossy."

The main differences, however, are in general as follows: the "smooth" (or glossy) colony is round, shining, translucent, reticular, and of smooth appearance, while the "rough" (or matt) colony is of an irregular or jagged contour, somewhat more opaque to transmitted light, and presents the appearance of a rough, matt surface.

Studies of these S and R forms showed no appreciable differences in their morphologic or biochemical reactions but very profound differences in other characteristics which may be summarized in tabular form as follows:[5]

SMOOTH TYPE	ROUGH TYPE
1. Emulsions stable in 0.85 per cent NaCl.	1. Agglutinated by 0.85 per cent NaCl.
2. Growth uniformly turbid in broth.	2. Growth forms sediment with clear supernatant fluid.
3. Motile (in the case of flagellated bacteria).	3. Motility often reduced or absent.
4. Agglutinated by specific serum in large flocculent clumps.	4. Agglutinated in small compact granules falling to the bottom of the tube.

These differences in the type of agglutination secured with S and R forms are of particular and practical importance in relation to diagnostic agglutination reactions and have been rather extensively studied, as a result of which rather definite information is accumulating.

O agglutination is granular, forms slowly, settles slowly, and presents flakes of fairly small and uniform size, leaving a clear supernatant fluid with a scanty sediment difficult to dislodge.

H agglutination, on the contrary, forms rapidly and settles rapidly, is flocculent in character, the flakes being in general large, though varying in size, and the supernatant fluid is cloudy while the sediment is voluminous and easily dislodged.

According to the observations of Weil and Felix[4] the agglutinins produced after inoculation with heat-killed vaccines are mainly of H type while those formed as a result of an actual attack of the disease are mainly of O type.

The practical importance of such a conclusion applied to diagnostic agglutination reactions has very naturally led to many studies.

Eldering and Larkum[6] from a study of 419 tests conducted with both O (alcohol-killed) and H (formalin-killed) antigen suspensions found the assumption relatively accurate, residual O agglutinins being present in quantity only after actual infection, although, of course, it must be borne in mind that the type of agglutinins following vaccination will naturally be in some measure dependent upon the preponderance of O or H antigens in the vaccine used.

Other observations[7, 8] are also corroboratory of these conclusions so that, while further observations and the study of large series should still be carried

on, the formulation of the following tentative dicta seems justified with regard to the diagnosis of enteric fevers by means of agglutination reactions:

1. As it has been conclusively demonstrated that both H (flagellate) and O (somatic) agglutinins are produced in most cases of typhoid fever it is advisable that agglutination tests be conducted with both H (formalin-killed) and O (alcohol-killed) antigen suspensions.

Dulaney, Wickle, and Trigg[7] from their studies prefer 1 per cent formalinized suspensions because the concentration inhibits the O agglutination.

2. The titer of the O agglutination is of definite value although further investigations will probably be required to determine the exact status of low titer reactions, concerning which there has been some discussion.

Felix originally suggested an agglutination titer of 1:100 or 1:200 as strongly suggestive if not diagnostic of infection. Dulaney, Wickle, and Trigg, however, finding such titers exceeded by 23 per cent of their nontyphoid cases, suggest that the titer be raised to 1:500 before being regarded as diagnostic.

On the other hand others, among them Gilbert, Coleman, and Laviano[9] regard agglutination in a 1:80 titer of distinct significance.

3. From the foregoing it follows that all diagnostic agglutination tests should be quantitative in character and devised to determine the highest titer in which agglutination occurs which, indeed, has long been the custom of most laboratories and should be the rule in all.

While all that has been said thus far applies to the Widal reaction in its application to the study of typhoid fever, it is important to emphasize that in principle as well as practical application it applies also to all agglutination tests or reactions.

It is essential also to recognize that the term "enteric fever" must be recognized as a generic term and as a clinical rather than a bacteriologic entity. For it is now recognized that there are at least twelve species within the Salmonella group which, though more commonly associated with acute gastroenteritis, are likewise capable of producing a continued fever in man. In addition to these, experience shows that in a possible explanation of continued fever in the human being both undulant fever and tularemia must be remembered.

This is of practical importance as concerns the selection of antigen suspensions against which the serum should be tested, in the determination of which several factors must be taken into consideration. In addition to the typhoid group (T, A, and B) the routine inclusion of abortus suspensions will be found of value, while the use of a *B. tularense* suspension may either be routine, where this disease is encountered with any frequency or reserved for such cases in which this disease is regarded as a diagnostic possibility. This same principle applies also to the infrequently encountered *B. paratyphosus C* (in the United States, at least), as well as to *B. enteritidis* and the other occasional causes of enteric fever. While it is suggested above that paratyphoid A suspensions constitute one of the routine antigens, this infection is so infrequently encountered that its inclusion is a matter of choice.

From a consideration of the available evidence, and the literature on this subject is assuming quite respectable proportions, it appears that: The determination of both somatic (O) and flagellar (H) antigens is advisable; and that the determinations have their most definite value when they are quantitative.

The question is of interest not only to the laboratory worker by whom the determinations are made but as well to the clinician by whom they are requested and who, in the majority of instances, will be responsible for their interpretation and clinical application.

The interpretation of agglutination reactions is governed by many factors in addition to those already discussed.

As agglutinins in enteric fever do not reach their maximal titer as a rule before the sixteenth to the twenty-first day, although they usually begin to appear during the first week, it follows that a negative reaction during the first week is of little diagnostic significance. It is not, however, valueless because it affords a valuable base line for comparison of any future fluctuations which may occur.

While, with repeated tests, 98 per cent of typhoid fever cases show the presence of agglutinins at some time during the course of the disease, and while, when both O and H antigens are used, the absence of a significant rise in the titer is exceptional, the incidence of positive reactions on a single test is accepted as that laid down by Park and Williams, namely: first week, 20 per cent; second week, 60 per cent; third week, 80 per cent; fourth week, 90 per cent.

In the interpretation of single reactions one must also take into account the frequency and concentration of H and O agglutinins both in the serum of the normal population and in subgroups differentiated by some particular experience, such as past epidemics or antityphoid vaccination. This, of course, varies in different localities.

It appears, however, that O agglutinins in low titer (1:20 to 1:50) are more common than H agglutinins in the normal nonvaccinated population, and likewise that T.A.B. vaccination results in a higher titer of H than O agglutinins.

In general, therefore, it may be concluded that a reaction of 1:80 with an alcohol-treated (O) antigen suspension usually suggests typhoid fever or an infection with a closely allied bacterial species, while a similar reaction with a formalinized (H) antigen suspension suggests several alternatives: that the patient has typhoid fever; that he has had the disease in the past; or that he has been vaccinated.

The principles governing the interpretation of agglutination reactions in enteric fever have been well summarized by Topley[10] and are here paraphrased:

1. A negative reaction to all the antigen suspensions used for the test may signify: (a) that the patient does not have enteric fever; (b) that the specimen has been taken before agglutinins have appeared in the blood; or, (c) that the enteric fever is of unusual type and caused by an organism not represented in the suspensions employed in the test.

The first two alternatives are differentiated by a repetition of the test at suitable intervals; the third requires further investigation directed especially toward detection of the organism by culture.

A negative reaction in the second or third week of the illness is of definite negative diagnostic value and repeated negative reactions render the correctness of the clinical diagnosis unlikely.

2. A positive reaction against any suspension must be considered in terms of: (a) the distribution of agglutinins among the normal population; (b) the stage of the disease at which the test was made; and (c) the history as regards vaccination.

A positive reaction of 1:40 or over in a nonvaccinated individual is of definite significance and probably not an expression of "normal" agglutinins.

3. As is the case with many other laboratory procedures, the trend of a series of agglutination tests is often of more value than a single reaction, especially in the case of those who have had antityphoid vaccination, any significant rise from the original titer having a decided significance.

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—*R. A. K.*